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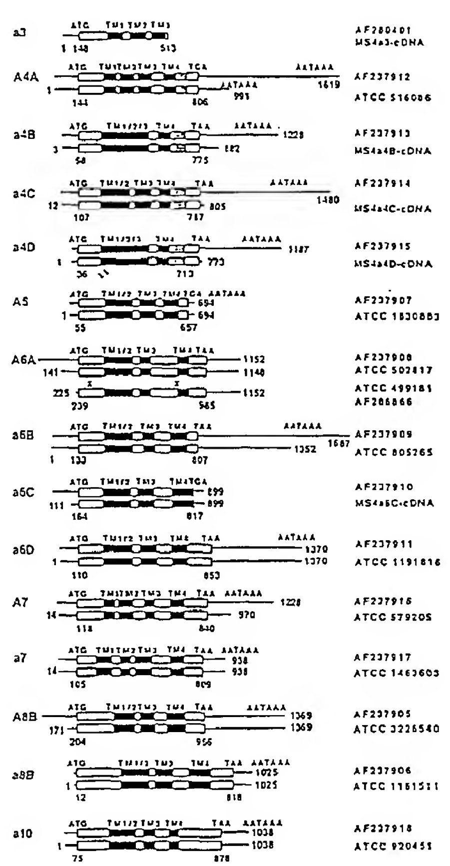
(54) Title: IDENTIFICATION OF NOVEL MS4A GENE FAMILY MEMBERS EXPRESSED BY HEMATOPOIETIC CELLS



(57) Abstract: Isolated nucleic acids encoding MS4A polypeptides, isolated MS4A polypeptides, and uses thereof. The disclosed MS4A nucleic acids and polypeptides can be used to generate a mouse model of atopic disorders, for drug discovery screens, and for therapeutic treatment of atopic disorders or other MS4A-related conditions.

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Description

IDENTIFICATION OF NOVEL MS4A GENE FAMILY MEMBERS EXPRESSED BY HEMATOPOIETIC CELLS

Cross Reference to Related Applications

This application is based on and claims priority to United States Provisional Application Serial Number 60/254,362, filed December 8, 2000, and United States Provisional Application Serial No. 60/270,057 filed February 20, 2001, herein incorporated by reference in their entirety.

Grant Statement

This work was supported by NIH grants CA-81776 and CA-54464. Thus, the U.S. Government has rights in the invention.

Field of the Invention

The present invention generally relates to a new class of MS4A proteins characterized by a membrane-embedded structure. More particularly, the present invention provides MS4A nucleic acid and polypeptide sequences, chimeric genes comprising disclosed MS4A sequences, antibodies that specifically recognize MS4A polypeptides, and uses thereof.

Table of Abbreviations

ATCC	American Tissue Culture Collection
CD20	CD20 B lymphocyte differentiation antigen
FcεRIβ	high-affinity IgE receptor β chain
GFP	green fluorescent protein
htgs	GenBank human genomic database
HTm4	hematopoietic CD20-like antigen
MS4A family	membrane spanning 4-domain family, subfamily A

Background Art

CD20, FcεRIβ, and HTm4 are three cell surface proteins expressed by hematopoietic cells that represent members of a nascent gene family (Adra et al. (1999) *Clin Genet* 55:431-437, Kinet (1999) *Annu Rev Immunol* 17:931-972; Tedder and Engel (1994) *Immunol Today* 15:450-454). The deduced amino acid sequence of human and mouse CD20 first demonstrated a cell surface protein containing four membrane-spanning regions, N– and C-terminal cytoplasmic domains, and an ~50 amino acid loop that serves as the extracellular domain (Einfeld et al. (1988) *EMBO J* 7:711-717; Stamenkovic and Seed (1988) *J Exp Med* 167:1975-1980; Tedder et al. (1988a) *J Immunol* 141:4388-4394; Tedder et al. (1988b) *Proc Natl Acad Sci USA* 85:208-212). Human CD20 shares 20% amino acid sequence identity with FcεRIβ and HTm4 (Adra et al. (1994) *Proc Natl Acad Sci USA* 91:10178-10182, Küster et al. (1992) *J Biol Chem* 267:12782-12787). Moreover, these three proteins have a similar overall structure in man, mouse, and rat with significant sequence identity within the first three membrane-spanning domains (Kinet et al. (1988) *Proc Natl Acad Sci USA* 85:6483-6487; Ra et al. (1989) *Nature* 19:1771-7; Tedder et al., 1988a). In addition, all three genes are located in the same region of human chromosome 11q12-13.1 (Adra et al., 1994; Hupp et al. (1989) *J Immunol* 143:3787-3791; Tedder et al. (1989a) *J Immunol* 142:2555-2559) and mouse chromosome 19 (Hupp et al. 1989; Tedder et al., 1988a). These three genes are therefore likely to have evolved from a common precursor.

Despite structural and sequence conservation between CD20, FcεRIβ and HTm4, transcription of each gene is differentially regulated. CD20 is only expressed by B lymphocytes (Stashenko et al. (1980) *J Immunol* 125:1678-1685; Tedder et al., 1988a). FcεRIβ is expressed by mast cells and basophils (Kinet, 1999). HTm4 is expressed by diverse lymphoid and myeloid origin hematopoietic cells (Adra et al., 1994).

Although the function of HTm4 remains unexplored, CD20 and FcεRIβ have critical roles in cell signaling. CD20 forms a homo- or hetero-tetrameric complex that is functionally important for regulating cell cycle progression

and signal transduction in B lymphocytes (Tedder and Engel, 1994). CD20 additionally regulates transmembrane Ca^{++} conductance, possibly as a functional component of a Ca^{++}-permeable cation channel (Bubien et al. *J Cell Biol* 121:1121-1132; Kanzaki et al. (1997a) *J Biol Chem* 272:14733-14739; Kanzaki et al. (1997b) *J Biol Chem* 272:4964-4969; Kanzaki et al. (1995) *J Biol Chem* 270:13099-13104). FcεRIβ is part of a tetrameric receptor complex consisting of α, β, and two γ chains (Blank et al. (1989) *Nature* 337:187-189). FcεRIβ mediates interactions with IgE-bound antigens that lead to cellular responses such as the degranulation of mast cells. Specifically, the FcεRIβ subunit functions as an amplifier of FcεRIβ-mediated activation signals (Dombrowicz et al. (1998) *Immunity* 8:517-529; Lin et al. (1996) *Cell* 85:985-995). Because of their unique structure and sequence homology, CD20, FcεRIβ, and HTm4 are likely to share overlapping functional properties.

CD20 and FcεRIβ are also important clinically. Antibodies against CD20 are effective in treating non-Hodgkin's lymphoma (McLaughlin et al. (1998) *Oncology* 12:1763-1769; Onrust et al. (1989) *J Biol Chem* 264:15323-15327; Weiner (1999) *Semin Oncol* 26:43-51). Genetic variations at chromosome 11q12-13 can also play a role in the pathogenesis of allergic diseases (Adra et al., 1999; Kinet, 1999). Recent studies suggest that FcεRIβ contributes to such diseases, and other genetic elements in this region likely also contribute to allergic disease.

Since CD20, FcεRIβ, and HTm4 are likely to have evolved by duplication of an ancestral gene, other related proteins might exist that form additional receptor complexes. In view of the clinical importance noted above, the identification of such proteins thus represents a long-felt and ongoing need in the art. To address this need, applicants have identified novel human and mouse proteins that span the cell membrane at least four times and share high levels of amino acid sequence identity with CD20, FcεRIβ, and HTm4. This finding reveals a new gene family that has been designated herein as the MS4A family (membrane spanning 4-domain family, subfamily A). Currently this family contains at least 10 subgroups

(MS4A1 through MS4A12) that encode at least 21 previously unidentified human and mouse proteins expressed by hematopoietic cells and by diverse cell types in non-hematopoietic tissues.

Summary of the Invention

The present invention discloses isolated MS4A polypeptides and isolated nucleic acid molecules encoding the same. Preferably, an isolated MS4A polypeptide, or functional portion thereof, comprises a polypeptide encoded by the nucleic acid molecule of any one of the odd numbered SEQ ID NOs:1-37 a polypeptide encoded by a nucleic acid molecule that is substantially identical to any one of the odd-numbered SEQ ID NOs:1-37, a polypeptide fragment encoded by a 20 nucleotide sequence that is identical to a contiguous 20 nucleotide sequence of any one of the odd-numbered SEQ ID NOs:1-37, a polypeptide having an amino acid sequence of any one of the even-numbered SEQ ID NOs:2-38, a polypeptide that is a biological equivalent of any one of the even-numbered SEQ ID NOs:2-38, or a polypeptide that is immunologically cross-reactive with an antibody that shows specific binding with a polypeptide comprising some or all amino acids of any one of the even-numbered SEQ ID NOs:2-38.

The present invention further teaches chimeric genes having a heterologous promoter that drives expression of a nucleic acid sequence encoding a MS4A polypeptide. Preferably, the chimeric gene is carried in a vector and introduced into a host cell so that a MS4A polypeptide of the present invention is produced. Preferred host cells include but are not limited to a bacterial cell, a hamster cell, a mouse cell, or a human cell.

In another aspect of the invention, a method is provided for detecting a nucleic acid molecule that encodes a MS4A polypeptide. According to the method, a biological sample having nucleic acid material is hybridized under stringent hybridization conditions to a MS4A nucleic acid molecule of the present invention. Such hybridization enables a nucleic acid molecule of the biological sample and the MS4A nucleic acid molecule to form a detectable duplex structure. Preferably, the MS4A nucleic acid molecule includes some

or all nucleotides of any one of the odd-numbered SEQ ID NOs:1-37. Also preferably, the biological sample comprises human nucleic acid material.

The present invention further teaches an antibody that specifically recognizes a MS4A polypeptide. Preferably, the antibody recognizes some or all amino acids of any one of the even-numbered SEQ ID NOs:2-38. A method for producing a MS4A antibody is also disclosed, and the method comprises recombinantly or synthetically producing a MS4A polypeptide, or portion thereof; formulating the MS4A polypeptide so that it is an effective immunogen; immunizing an animal with the formulated polypeptide to generate an immune response that includes production of MS4A antibodies; and collecting blood serum from the immunized animal containing antibodies that specifically recognize a MS4A polypeptide. Antibody-producing cells can be optionally fused with an immortal cell line whereby a monoclonal antibody that specifically recognizes a MS4A polypeptide can be selected. Preferably, the MS4A polypeptide used as an immunogen includes some or all amino acid sequences of any one the even-numbered SEQ ID NOs:2-38.

A method is also provided for detecting a level of MS4A polypeptide using an antibody that specifically recognizes a MS4A polypeptide. According to the method, a biological sample is obtained from an experimental subject and a control subject, and a MS4A polypeptide is detected in the sample by immunochemical reaction with the MS4A antibody. Preferably, the antibody recognizes amino acids of any one of the even-numbered SEQ ID NOs:2-38, and is prepared according to a method of the present invention for producing such an antibody.

The present invention further discloses a method for identifying a compound that modulates MS4A function. The method comprises: exposing an isolated MS4A polypeptide to one or more compounds, and assaying binding of a compound to the isolated MS4A polypeptide. A compound is selected that demonstrates specific binding to the isolated MS4A polypeptide. Preferably, the MS4A polypeptide used in the binding assay of the method includes some or all amino acids of any one of the even-numbered SEQ ID NOs:2-38.

Also provided is a method for identifying a regulator of MS4A gene expression. The method comprises (a) exposing a cell sample with a candidate compound to be tested, the cell sample containing at least one cell containing a DNA construct comprising a modulatable transcriptional regulatory sequence of a MS4A-encoding nucleic acid and a reporter gene which is capable of producing a detectable signal; (b) evaluating an amount of signal produced in relation to a control sample; and (c) identifying a candidate compound as a modulator of MS4A gene expression based on the amount of signal produced in relation to a control sample. Preferably, the modulatable transcriptional regulatory sequence of a MS4A-encoding nucleic acid comprises a sequence that is immediately upstream of the initial coding region of a MS4A gene as set forth in any one of SEQ ID NOs:73-81.

The present invention further provides a method for modulating MS4A function in a subject. According to the method, a pharmaceutical composition is prepared that includes a substance capable of modulating MS4A expression or function, and a carrier. An effective dose of the pharmaceutical composition is administered to a subject, whereby MS4A activity is altered in the subject. Provided are therapeutic methods wherein a change in MS4A activity comprises a shift in the abundance of cell subpopulations expressing said protein, modulation of $[Ca^{2+}]_i$ levels, or altered cell function. In a preferred embodiment, the substance used to perform this method shows specific binding to some or all amino acids of any one of the even-numbered SEQ ID NOs:2-38, and was discovered by a method of the present invention. In another embodiment, MS4A function is disrupted by immunizing a subject with an effective dose of the disclosed MS4A polypeptide. The immune system of the subject produces an antibody that specifically recognizes the MS4A polypeptide, and preferably recognizes some or all of amino acids of any one of the even-numbered SEQ ID NOs:2-38. In a further embodiment, a gene therapy vector is used, the vector comprising a nucleotide sequence encoding a MS4A polypeptide. Alternatively, the gene therapy vector comprises a nucleotide sequence encoding a nucleic acid molecule, a peptide, or a protein that interacts with a

MS4A nucleic acid or polypeptide. Preferably, the subject is a human subject.

Accordingly, it is an object of the present invention to provide novel MS4A nucleic acid and polypeptide sequences, and novel methods relating thereto. This object is achieved in whole or in part by the present invention.

An object of the invention having been stated above, other objects and advantages of the present invention will become apparent to those skilled in the art after a study of the following description of the invention, Figures and non-limiting Examples.

Brief Description of the Drawings

Figure 1 depicts cDNAs encoded by fifteen new human or mouse MS4A gene products. Consensus sequences from cDNAs and overlapping ESTs are indicated by their GenBank Accession numbers. Representative full-length cDNAs for each gene product are shown, except for *MS4a3* which was not full-length. 5' and 3' untranslated sequences are shown as horizontal lines with relative nucleotide lengths shown. Coding regions are shown as boxes with translation initiation and termination codons and their relative nucleotide locations shown. Poly(A) attachment signal sequences (AATAAA) are indicated when known. Deduced hydrophobic regions are shown as filled boxes with the predicted membrane-spanning domains shown as TM1-TM4. Additional hydrophobic regions in MS4A4 proteins are shown as shaded boxes. Sites of putative nucleotide polymorphisms in *MS4A6A* are indicated by two (X)s.

Figure 2 depicts exon-intron organization of the human MS4A genes. The maps were constructed by aligning known and predicted MS4A cDNA sequences with human genomic sequences as described in Materials and Methods. Exons are shown as boxes with the predicted translation initiation codons (ATG), transmembrane domains (TM) and termination codons indicated on the top. All exon and intron distances are shown to scale. Gaps indicate where intron distances have not been determined for *MS4A3*, *MS4A4A*, and *MS4A12*. Two long introns present in *MS4A4E* are not to

scale but the intron lengths are indicated. Exon numbering for *MS4A1*, and *MS4A2* is as published (Küster et al., 1992; Tedder et al., 1988a; Tedder et al., 1988b).

Figure 3 shows human MS4A4E protein and transcript sequences predicted from genomic DNA sequences. *MS4A4E* sequences are compared with human *MS4A4A* cDNA (disclosed herein) and genomic sequences. Gaps were introduced to provide optimal alignment. The boxed AAC sequence near the 5' end of the *MS4A4A* sequence indicates the length of the most 5' *MS4A4A* cDNA sequence. Sequences upstream of this are based on contiguous genomic DNA sequences. Nucleotide numbering is based on the *MS4A4A* cDNA sequence, disclosed herein. Predicted translation initiation codons are shaded. Predicted membrane-spanning regions are underlined. An asterisk indicates predicted translation termination codons. Potential poly-A attachment signal sequences (AATAAA) are boxed.

Figure 4 shows human MS4A6E protein and transcript sequences predicted from genomic DNA and overlapping cDNA sequences. Predicted*MS4A6E* transcript sequences are compared with human *MS4A6A* cDNA sequence (disclosed herein). Gaps were introduced in the nucleotide sequence to provide optimal alignment. The 5' end of both transcripts start at 3' splice-acceptor sites which demark the first translated exons for both genes. The 5' end of the putative *MS4A6E* transcript is based on genomic DNA sequence, while the predicted sequences starting at nucleotide 60 were based on both genomic DNA sequences and overlapping cDNA sequences. A gap in the *MS4A6A* sequence is indicated where TM1/2 and TM2 exons are not found in *MS4A6E* transcripts. *MS4A6A* nucleotide numbering is based on the cDNA sequence (disclosed herein). Predicted translation initiation codons are shaded. Predicted membrane-spanning regions are underlined. An asterisk indicates the predicted translation termination codon for the MS4A6E protein.

Figure 5 shows human MS4A10 protein and transcript sequences predicted from human genomic DNA sequences. *MS4A10* nucleotide

sequence is compared with mouse *MS4a10* cDNA sequence (disclosed herein). The 5' end of both transcripts start at 3' splice-acceptor sites which demark the first translated exons for both genes. *MS4a10* nucleotide numbering is based on the cDNA sequence (disclosed herein). Predicted translation initiation codons are shaded. Predicted membrane-spanning regions are underlined. An asterisk indicates predicted translation termination codon for the MS4A10 protein. Potential poly-A attachment signal sequences (AATAAA) are boxed.

Figure 6 depicts a physical linkage map for the MS4A genes. A scheme for chromosome 11 structure is shown on the left with the mapped locations for *MS4A1*, *MS4A2* and *MS4A3* indicated. Representative human BAC clones are shown as vertical black bars with clone names shown on the top and clone size shown at the bottom. All distances are shown to the indicated scale. The distance between and spatial relationship of RP11-312N17 to the four other overlapping BACs shown at the bottom are unknown. Thin bars indicate continuous characterized (mapped or sequenced) regions of DNA that contain identified MS4A genes. When the relative position of this region of DNA is known relative to the representative BACs that are shown, the thin bars overlay the BAC. The mapped position of each MS4A gene is indicated on the right with the relative direction of gene translation indicated by arrows (⟶). In some cases, approximate distances between MS4A genes (termination codons to the translation initiation codon for the next gene) are indicated in base pairs (bp). In some cases, approximate MS4A gene size is indicated showing the distance between predicted translation initiation codons and translation termination codons as show in Figure 7.

Figure 7 depicts deduced amino acid sequences for CD20 (human A1, SEQ ID NO:40; mouse a1, SEQ ID NO:48), FcεRIβ (human A2, SEQ ID NO:42; mouse a2, SEQ ID NO:50), HTm4 (human A3, SEQ ID NO:44; mouse a3, SEQ ID NO:20), and 19 new MS4A (human) (even-numbered SEQ ID NOs:2-18, 46) and MS4a (mouse and pig) proteins (even-numbered SEQ ID NOs:22-38, 56). Gaps were introduced to optimize alignments.

Numbers represent predicted residue positions. The predicted membrane-spanning regions (TM1-TM4) are indicated. Predicted intron|exon splice junctions are indicated by vertical bars where information was available. Amino acids common to 10 or more proteins are shaded. *indicates partial sequence for the MS4a3 protein. CD20, FcεRIβ, and HTm4 sequences and known intron|exon borders (SEQ ID NOs:39-44, 47-50) are as published (Adra et al., 1994; Küster et al., 1992; Ra et al., 1989; Tedder et al., 1988a; Tedder et al., 1989b; Tedder et al., 1988b). MS4A12 represents a conceptual translation (SEQ ID NO:46) of a human colon mucosa cDNA sequence (GenBank AK000224, SEQ ID NO:45), and MS4a12 represents a conceptual translation (SEQ ID NO:56) of a homologous cDNA sequence from pig (GenBank AJ236932, SEQ ID NO:55).

Figure 8 depicts UPGMA (unweighted pair group method using arithmetic averages) tree of deduced MS4A and MS4a protein sequences. Horizontal tree branch length is a measure of sequence relatedness. For example, MS4a4B and MS4a4C are the most similar in sequence, while CD20 (MS4A1) sequences were the most divergent from other family members. The MS4a12p sequence was from pig, while all other MS4a sequences were from mouse. The UPGMA tree was generated using Geneworks version 2.0 (IntelliGenetics, Inc., Mountain View, California, USA).

Figure 9 shows immunofluorescent detection of CD20 expression during B cell development. Single cell suspensions of leukocytes were isolated from wild-type mice, stained using MB20-13 (visualized using a PE-conjugated, anti-mouse IgG3 antibody) and anti-B220 (FITC-conjugated) monoclonal antibodies, and examined by two-color immunofluorescence staining with flow cytometry analysis. Quadrant gates indicate negative and positive populations of cells as determined using isotype-matched control monoclonal antibodies. The gated cell populations correspond to the cells described in Table 7, and are shown for reference. These results are representative of those obtained with six (6) two month-old wild type mice.

Figure 10 summarizes the strategy for targeted disruption of the mouse *CD20* gene.

Figure 10A shows genomic clones encoding CD20.

Figure 10B shows the intron-exon organization of the wild type*CD20* allele containing exons 5-8 (shaded squares).

Figure 10C shows the structure of the CD20 targeting vector.

Figure 10D shows the predicted structure of the CD20 allele after gene targeting in ES cells by homologous recombination. The EcoR V restriction site in exon 6 is deleted as indicated.

Figure 10E presents Southern blot analysis of tail DNA from two wild type and four $CD20^{-/-}$ mice. Genomic DNA was digested with EcoR V, transferred to nitrocellulose and hybridized with the 5' probe indicated in (D).

Figure 10F shows PCR amplification of genomic DNA from wild type and $CD20^{-/-}$ mice using primers that bind in exons 6 and 7. Amplification of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) is shown as a positive control.

Figure 10G shows PCR amplification of cDNA generated from splenic RNA of wild type and $CD20^{-/-}$ mice. Each reaction mixture contained a sense primer that hybridized with sequences encoded by exon 3 and antisense primers that hybridized with either exon 6 or *Neo*^{r} gene promoter sequences.

Figures 10H and 10I show reactivity of the MB20-13 monoclonal antibody with CD20 cDNA-transfected (thick line) or untransfected (dashed line) 300.19 cells (Figure 10H) or Chinese Hamster Ovary (CHO) cells (Figure 10I). The thin lines represent CD20 cDNA-transfected cells stained with secondary antibody alone or an isotype-control monoclonal antibody. Indirect immunofluorescence staining was visualized by flow cytometry analysis.

Figure 10J shows immunofluorescent staining of splenocytes from $CD20^{-/-}$ or wild type mice with MB20-13 (visualized using a PE-conjugated, anti-mouse IgG3 antibody) and anti-B220 (FITC-conjugated) monoclonal antibodies. Splenocytes from $CD20^{-/-}$ mice generated histograms identical to

those obtained without MB20-1 monoclonal antibody present, using the secondary antibody alone.

Figure 11 depicts immunofluorescent detection of B lymphocyte subpopulations in $CD20^{-/-}$ and wild type mice. Lymphocytes were isolated and examined by two color immunofluorescent staining with flow cytometry analysis. Quadrants delineated by squares indicate negative and positive populations of cells as determined using unreactive monoclonal antibody controls. The gated cell populations correspond to the cells described in Table 7 that represent at least 6 mice of each genotype.

Figure 12 shows altered signal transduction in $CD20^{-/-}$ B cells. Figure 12 also shows CD19 expression by splenocytes from $CD20^{-/-}$ (thin line) and wild type (thick line) mice. Immunofluorescence staining using PE-conjugated anti-CD19 monoclonal antibody with flow cytometry analysis. The dashed line represents staining of wild type splenocytes with a control antibody.

Figure 12A presents calcium responses induced by BCR or CD19 ligation in $CD20^{-/-}$ and wild type B cells. Splenocytes were loaded with 1 μM indo-1-AM ester and B cells were stained with FITC-conjugated anti-B220 antibody. At 1 min (arrow), optimal concentrations of goat anti-IgM $F(ab')_2$ antibody fragments, anti-CD19 monoclonal antibody or Thapsigargin were added, with or without EGTA present. Increased ratios of indo-1 fluorescence indicate increased $[Ca^{2+}]_i$. Results represent those from at least four experiments.

Figure 12B presents assays of tyrosine phosphorylation of proteins from purified splenic B cells of $CD20^{-/-}$ and wild type mice. B cells (2×10^7/sample) were incubated with anti-IgM antibody for the times shown and detergent lysed. Proteins were resolved by SDS-PAGE, transferred to nitrocellulose and immunoblotted with anti-phosphotyrosine (anti-PTyr) antibody. The blot was stripped and reprobed with anti-SHP-1 antibody as a control for equivalent protein loading. Western blots from two of three experiments are shown to demonstrate the range of results.

Detailed Description of the Invention

The present invention provides isolated nucleic acids encoding MS4A polypeptides (representative embodiments set forth as the odd-numbered SEQ ID NOs:1-37), isolated MS4A polypeptides (representative embodiments set forth as the even-numbered SEQ ID NOs:2-38), and uses thereof. The disclosed MS4A nucleic acids and polypeptides can be used according to methods of the present invention for drug discovery screens, for therapeutic treatment of atopic conditions, and for therapeutic regulation of $[Ca^{2+}]_i$ levels, among other uses.

I. DEFINITIONS

While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the invention. The entire contents of all publications mentioned herein, including the discussion of the background art presented above, are hereby fully incorporated by reference.

I.A. MS4A nucleic acids

The nucleic acid molecules provided by the present invention include the isolated nucleic acid molecules of any one of the odd-numbered SEQ ID NOs:1-37, sequences substantially similar to sequences of any one of the odd-numbered SEQ ID NOs:1-37, conservative variants thereof, subsequences and elongated sequences thereof, complementary DNA molecules, and corresponding RNA molecules. The present invention also encompasses genes, cDNAs, chimeric genes, and vectors comprising disclosed MS4A nucleic acid sequences.

The term "nucleic acid molecule" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides which have similar properties as the reference natural nucleic acid. Unless otherwise indicated, a particular nucleotide sequence also implicitly encompasses conservatively modified variants thereof (*e.g.* degenerate codon substitutions),

complementary sequences, subsequences, elongated sequences, as well as the sequence explicitly indicated. The terms “nucleic acid molecule” or “nucleotide sequence” can also be used in place of “gene”, “cDNA”, or “mRNA”. Nucleic acids can be derived from any source, including any organism.

The term “isolated”, as used in the context of a nucleic acid molecule, indicates that the nucleic acid molecule exists apart from its native environment and is not a product of nature. An isolated DNA molecule can exist in a purified form or can exist in a non-native environment such as a transgenic host cell.

The term “purified”, when applied to a nucleic acid, denotes that the nucleic acid is essentially free of other cellular components with which it is associated in the natural state. Preferably, a purified nucleic acid molecule is a homogeneous dry or aqueous solution. The term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the nucleic acid is at least about 50% pure, more preferably at least about 85% pure, and most preferably at least about 99% pure.

The term "substantially identical", the context of two nucleotide or amino acid sequences, can also be defined as two or more sequences or subsequences that have at least 60%, preferably 80%, more preferably 90-95%, and most preferably at least 99% nucleotide or amino acid sequence identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms (described herein below under the heading <u>Nucleotide and Amino Acid Sequence Comparisons</u>) or by visual inspection. Preferably, the substantial identity exists in nucleotide sequences of at least 50 residues, more preferably in nucleotide sequence of at least about 100 residues, more preferably in nucleotide sequences of at least about 150 residues, and most preferably in nucleotide sequences comprising complete coding sequences. In one aspect, polymorphic sequences can be substantially identical sequences. The term “polymorphic” refers to the occurrence of two or more

genetically determined alternative sequences or alleles in a population. An allelic difference can be as small as one base pair.

Another indication that two nucleotide sequences are substantially identical is that the two molecules specifically or substantially hybridize to each other under stringent conditions. In the context of nucleic acid hybridization, two nucleic acid sequences being compared can be designated a “probe” and a “target”. A “probe” is a reference nucleic acid molecule, and a "“target” is a test nucleic acid molecule, often found within a heterogenous population of nucleic acid molecules. A “target sequence” is synonymous with a “test sequence”.

A preferred nucleotide sequence employed for hybridization studies or assays includes probe sequences that are complementary to or mimic at least an about 14 to 40 nucleotide sequence of a nucleic acid molecule of the present invention. Preferably, probes comprise 14 to 20 nucleotides, or even longer where desired, such as 30, 40, 50, 60, 100, 200, 300, or 500 nucleotides or up to the full length of any of those set forth as the odd-numbered SEQ ID NOs:1-37. Such fragments can be readily prepared by, for example, directly synthesizing the fragment by chemical synthesis, by application of nucleic acid amplification technology, or by introducing selected sequences into recombinant vectors for recombinant production.

The phrase "hybridizing specifically to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex nucleic acid mixture (*e.g.,* total cellular DNA or RNA). The phrase “binds substantially to” refers to complementary hybridization between a probe nucleic acid molecule and a target nucleic acid molecule and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired hybridization.

"Stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and Northern blot analysis are both sequence- and environment-dependent. Longer sequences hybridize specifically at higher temperatures.

An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes, part I chapter 2, Elsevier, New York, New York. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. Typically, under "stringent conditions" a probe will hybridize specifically to its target subsequence, but to no other sequences.

The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of stringent hybridization conditions for Southern or Northern Blot analysis of complementary nucleic acids having more than about 100 complementary residues is overnight hybridization in 50% formamide with 1 mg of heparin at 42°C. An example of highly stringent wash conditions is 15 minutes in 0.1 5 M NaCl at 65°C. An example of stringent wash conditions is 15 minutes in 0.2X SSC buffer at 65°C (See Sambrook et al. eds. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example of medium stringency wash conditions for a duplex of more than about 100 nucleotides, is 15 minutes in 1X SSC at 45°C. An example of low stringency wash for a duplex of more than about 100 nucleotides, is 15 minutes in 4-6X SSC at 40°C. For short probes (*e.g.,* about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less than about 1.0 M Na^+ ion, typically about 0.01 to 1.0 M Na^+ ion concentration (or other salts) at pH 7.0-8.3, and the temperature is typically at least about 30°C. Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. In general, a signal to noise ratio of 2-fold (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization.

The following are examples of hybridization and wash conditions that can be used to clone homologous nucleotide sequences that are substantially identical to reference nucleotide sequences of the present invention: a probe nucleotide sequence preferably hybridizes to a target nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C followed by washing in 2X SSC, 0.1% SDS at 50°C; more preferably, a probe and target sequence hybridize in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C followed by washing in 1X SSC, 0.1% SDS at 50°C; more preferably, a probe and target sequence hybridize in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C followed by washing in 0.5X SSC, 0.1% SDS at 50°C; more preferably, a probe and target sequence hybridize in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C followed by washing in 0.1X SSC, 0.1% SDS at 50°C; more preferably, a probe and target sequence hybridize in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C followed by washing in 0.1X SSC, 0.1% SDS at 65°C.

A further indication that two nucleic acid sequences are substantially identical is that proteins encoded by the nucleic acids are substantially identical, share an overall three-dimensional structure, are biologically functional equivalents, or are immunologically cross-reactive. These terms are defined further under the heading MS4A Polypeptides herein below. Nucleic acid molecules that do not hybridize to each other under stringent conditions are still substantially identical if the corresponding proteins are substantially identical. This can occur, for example, when two nucleotide sequences are significantly degenerate as permitted by the genetic code.

The term "conservatively substituted variants" refers to nucleic acid sequences having degenerate codon substitutions wherein the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al. (1991) *Nucleic Acids Res* 19:5081; Ohtsuka et al. (1985) *J Biol Chem* 260:2605-2608; Rossolini et al. (1994) *Mol Cell Probes* 8:91-98).

The term “subsequence” refers to a sequence of nucleic acids that comprises a part of a longer nucleic acid sequence. An exemplary subsequence is a probe, described herein above, or a primer. The term "primer" as used herein refers to a contiguous sequence comprising about 8 or more deoxyribonucleotides or ribonucleotides, preferably 10-20 nucleotides, and more preferably 20-30 nucleotides of a selected nucleic acid molecule. The primers of the invention encompass oligonucleotides of sufficient length and appropriate sequence so as to provide initiation of polymerization on a nucleic acid molecule of the present invention.

The term “elongated sequence” refers to an addition of nucleotides (or other analogous molecules) incorporated into the nucleic acid. For example, a polymerase (e.g., a DNA polymerase), e.g., a polymerase which adds sequences at the 3' terminus of the nucleic acid molecule. In addition, the nucleotide sequence can be combined with other DNA sequences, such as promoters, promoter regions, enhancers, polyadenylation signals, intronic sequences, additional restriction enzyme sites, multiple cloning sites, and other coding segments.

The term “complementary sequence”, as used herein, indicates two nucleotide sequences that comprise antiparallel nucleotide sequences capable of pairing with one another upon formation of hydrogen bonds between base pairs. As used herein, the term “complementary sequences” means nucleotide sequences which are substantially complementary, as can be assessed by the same nucleotide comparison set forth above, or is defined as being capable of hybridizing to the nucleic acid segment in question under relatively stringent conditions such as those described herein. A particular example of a complementary nucleic acid segment is an antisense oligonucleotide.

The term “gene” refers broadly to any segment of DNA associated with a biological function. A gene encompasses sequences including but not limited to a coding sequence, a promoter region, a cis-regulatory sequence, a non-expressed DNA segment is a specific recognition sequence for regulatory proteins, a non-expressed DNA segment that contributes to gene

expression, a DNA segment designed to have desired parameters, or combinations thereof. A gene can be obtained by a variety of methods, including cloning from a biological sample, synthesis based on known or predicted sequence information, and recombinant derivation of an existing sequence.

The term “gene expression” generally refers to the cellular processes by which a biologically active polypeptide is produced from a DNA sequence.

The present invention also encompasses chimeric genes comprising the disclosed MS4A sequences. The term “chimeric gene”, as used herein, refers to a promoter region operably linked to a MS4A coding sequence, a nucleotide sequence producing an antisense RNA molecule, a RNA molecule having tertiary structure, such as a hairpin structure, or a double-stranded RNA molecule.

The term “operably linked”, as used herein, refers to a promoter region that is connected to a nucleotide sequence in such a way that the transcription of that nucleotide sequence is controlled and regulated by that promoter region. Techniques for operatively linking a promoter region to a nucleotide sequence are well known in the art.

The terms "heterologous gene", “heterologous DNA sequence”, "heterologous nucleotide sequence", "exogenous nucleic acid molecule", or “exogenous DNA segment”, as used herein, each refer to a sequence that originates from a source foreign to an intended host cell or, if from the same source, is modified from its original form. Thus, a heterologous gene in a host cell includes a gene that is endogenous to the particular host cell but has been modified, for example by mutagenesis or by isolation from native cis-regulatory sequences. The terms also include non-naturally occurring multiple copies of a naturally occurring nucleotide sequence. Thus, the terms refer to a DNA segment that is foreign or heterologous to the cell, or homologous to the cell but in a position within the host cell nucleic acid wherein the element is not ordinarily found.

The term “promoter region” defines a nucleotide sequence within a

gene that is positioned 5' to a coding sequence of a same gene and functions to direct transcription of the coding sequence. The promoter region includes a transcriptional start site and at least one cis-regulatory element. The present invention encompasses nucleic acid sequences that comprise a promoter region of a MS4A gene, or functional portion thereof.

The term “cis-acting regulatory sequence” or “cis-regulatory motif” or “response element”, as used herein, each refer to a nucleotide sequence that enables responsiveness to a regulatory transcription factor. Responsiveness can encompass a decrease or an increase in transcriptional output and is mediated by binding of the transcription factor to the DNA molecule comprising the response element.

The term “transcription factor” generally refers to a protein that modulates gene expression by interaction with the cis-regulatory element and cellular components for transcription, including RNA Polymerase, Transcription Associated Factors (TAFs), chromatin-remodeling proteins, and any other relevant protein that impacts gene transcription.

A “functional portion” of a promoter gene fragment is a nucleotide sequence within a promoter region that is required for normal gene transcription. To determine nucleotide sequences that are functional, the expression of a reporter gene is assayed when variably placed under the direction of a promoter region fragment.

Promoter region fragments can be conveniently made by enzymatic digestion of a larger fragment using restriction endonucleases or DNAse I. Preferably, a functional promoter region fragment comprises about 5000 nucleotides, more preferably 2000 nucleotides, more preferably about 1000 nucleotides. Even more preferably a functional promoter region fragment comprises about 500 nucleotides, even more preferably a functional promoter region fragment comprises about 100 nucleotides, and even more preferably a functional promoter region fragment comprises about 20 nucleotides.

The terms “reporter gene” or “marker gene” or “selectable marker” each refer to a heterologous gene encoding a product that is readily

observed and/or quantitated. A reporter gene is heterologous in that it originates from a source foreign to an intended host cell or, if from the same source, is modified from its original form. Non-limiting examples of detectable reporter genes that can be operably linked to a transcriptional regulatory region can be found in Alam & Cook (1990) *Anal Biochem* 188:245-254 and PCT International Publication No. WO 97/47763. Preferred reporter genes for transcriptional analyses include the *lacZ* gene (<u>See</u>, *e.g.*, Rose & Botstein (1983) *Meth Enzymol* 101:167-180), Green Fluorescent Protein (GFP) (Cubitt et al. (1995) *Trends Biochem Sci* 20:448-455), luciferase, or chloramphenicol acetyl transferase (CAT). Preferred reporter genes for methods to produce transgenic animals include but are not limited to antibiotic resistance genes, and more preferably the antibiotic resistance gene confers neomycin resistance. Any suitable reporter and detection method can be used, and it will be appreciated by one of skill in the art that no particular choice is essential to or a limitation of the present invention.

An amount of reporter gene can be assayed by any method for qualitatively or preferably, quantitatively determining presence or activity of the reporter gene product. The amount of reporter gene expression directed by each test promoter region fragment is compared to an amount of reporter gene expression to a control construct comprising the reporter gene in the absence of a promoter region fragment. A promoter region fragment is identified as having promoter activity when there is significant increase in an amount of reporter gene expression in a test construct as compared to a control construct. The term "significant increase", as used herein, refers to an quantified change in a measurable quality that is larger than the margin of error inherent in the measurement technique, preferably an increase by about 2-fold or greater relative to a control measurement, more preferably an increase by about 5-fold or greater, and most preferably an increase by about 10-fold or greater.

The present invention further includes vectors comprising the disclosed MS4A sequences, including plasmids, cosmids, and viral vectors.

The term “vector”, as used herein refers to a DNA molecule having sequences that enable its replication in a compatible host cell. A vector also includes nucleotide sequences to permit ligation of nucleotide sequences within the vector, wherein such nucleotide sequences are also replicated in a compatible host cell. A vector can also mediate recombinant production of a MS4A polypeptide, as described further herein below. Preferred vectors include but are not limited to pBluescript (Stratagene), pUC18, pBLCAT3 (Luckow & Schutz (1987) *Nucleic Acids Res* 15:5490), pLNTK (Gorman et al. (1996) *Immunity* 5:241-252), and pBAD/gIII (Stratagene). A preferred host cell is a mammalian cell; more preferably the cell is a Chinese hamster ovary cell, a HeLa cell, a baby hamster kidney cell, or a mouse cell; even more preferably the cell is a human cell.

Nucleic acids of the present invention can be cloned, synthesized, recombinantly altered, mutagenized, or combinations thereof. Standard recombinant DNA and molecular cloning techniques used to isolate nucleic acids are well known in the art. Exemplary, non-limiting methods are described by Sambrook et al., eds. (1989); by Silhavy et al. (1984) <u>Experiments with Gene Fusions</u>, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; by Ausubel *et al.* (1992) <u>Current Protocols in Molecular Biology</u>, John Wylie and Sons, Inc., New York, New York; and by Glover, ed. (1985) <u>DNA Cloning: A Practical Approach</u>, MRL Press, Ltd., Oxford, United Kingdom. Site-specific mutagenesis to create base pair changes, deletions, or small insertions are also well known in the art as exemplified by publications, <u>see</u>, *e.g.*, Adelman et al., (1983) *DNA* 2:183; Sambrook et al. (1989).

Sequences detected by methods of the invention can be detected, subcloned, sequenced, and further evaluated by any measure well known in the art using any method usually applied to the detection of a specific DNA sequence including but not limited to dideoxy sequencing, PCR, oligomer restriction (Saiki et al. (1985) *Bio/Technology* 3:1008-1012), allele-specific oligonucleotide (ASO) probe analysis (Conner et al. (1983) *Proc Natl Acad Sci USA* 80:278), and oligonucleotide ligation assays (OLAs) (Landgren et.

al. (1988) *Science* 241:1007). Molecular techniques for DNA analysis have been reviewed (Landgren et. al. (1988) *Science* 242:229-237).

I.B. MS4A Polypeptides

The polypeptides provided by the present invention include the isolated polypeptides set forth as the even-numbered SEQ ID NOs:2-38, polypeptides substantially similar to the even-numbered SEQ ID NOs:2-38, MS4A polypeptide fragments, fusion proteins comprising MS4A amino acid sequences, biologically functional analogs, and polypeptides that cross-react with an antibody that specifically recognizes a MS4A polypeptide.

The term “isolated”, as used in the context of a polypeptide, indicates that the polypeptide exists apart from its native environment and is not a product of nature. An isolated polypeptide can exist in a purified form or can exist in a non-native environment such as, for example, in a transgenic host cell.

The term “purified”, when applied to a polypeptide, denotes that the polypeptide is essentially free of other cellular components with which it is associated in the natural state. Preferably, a polypeptide is a homogeneous solid or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A polypeptide which is the predominant species present in a preparation is substantially purified. The term "purified" denotes that a polypeptide gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the polypeptide is at least about 50% pure, more preferably at least about 85% pure, and most preferably at least about 99% pure.

The term “substantially identical” in the context of two or more polypeptides sequences is measured by (a) polypeptide sequences having about 35%, or 45%, or preferably from 45-55%, or more preferably 55-65%, or most preferably 65% or greater amino acids which are identical or functionally equivalent. Percent “identity” and methods for determining identity are defined herein below under the heading Nucleotide and Amino Acid Sequence Comparisons.

Substantially identical polypeptides also encompass two or more polypeptides sharing a conserved three-dimensional structure. Computational methods can be used to compare structural representations, and structural models can be generated and easily tuned to identify similarities around important active sites or ligand binding sites. See Henikoff et al. (2000) *Electrophoresis* 21(9):1700-1706; Huang et al. (2000) *Pac Symp Biocomput* 230-241; Saqi et al. (1999) *Bioinformatics* 15(6):521-522; and Barton (1998) *Acta Crystallogr D Biol Crystallogr* 54:1139-1146.

The term "functionally equivalent" in the context of amino acid sequences is well known in the art and is based on the relative similarity of the amino acid side-chain substituents. See Henikoff & Henikoff (2000) *Adv Protein Chem* 54:73-97. Relevant factors for consideration include side-chain hydrophobicity, hydrophilicity, charge, and size. For example, arginine, lysine, and histidine are all positively charged residues; that alanine, glycine, and serine are all of similar size; and that phenylalanine, tryptophan, and tyrosine all have a generally similar shape. By this analysis, described further herein below, arginine, lysine, and histidine; alanine, glycine, and serine; and phenylalanine, tryptophan, and tyrosine; are defined herein as biologically functional equivalents.

In making biologically functional equivalent amino acid substitutions, the hydropathic index of amino acids can be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+ 4.5); valine (+ 4.2); leucine (+ 3.8); phenylalanine (+ 2.8); cysteine (+ 2.5); methionine (+ 1.9); alanine (+ 1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte et al. (1982) *J Mol Biol* 157:105.). It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index

or score and still retain a similar biological activity. In making changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indices are within ±2 of the original value is preferred, those which are within ±1 of the original value are particularly preferred, and those within ±0.5 of the original value are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent No. 4,554,101 states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.* with a biological property of the protein. It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent protein.

As detailed in U.S. Patent No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+ 3.0); lysine (+ 3.0); aspartate (+ 3.0±1); glutamate (+ 3.0±1); serine (+ 0.3); asparagine (+ 0.2); glutamine (+ 0.2); glycine (0); threonine (-0.4); proline (-0.5±1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4) .

In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ±2 of the original value is preferred, those which are within ±1 of the original value are particularly preferred, and those within ±0.5 of the original value are even more particularly preferred.

The present invention also encompasses MS4A polypeptide fragments or functional portions of a MS4A polypeptide. Such functional portion need not comprise all or substantially all of the amino acid sequence of a native MS4A gene product. The term "functional" includes any biological activity or feature of MS4A, including immunogenicity.

The present invention also includes longer sequences of a MS4A polypeptide, or portion thereof. For example, one or more amino acids can

be added to the N-terminus or C-terminus of a MS4A polypeptide. Fusion proteins comprising MS4A polypeptide sequences are also provided within the scope of the present invention. Methods of preparing such proteins are known in the art.

The present invention also encompasses functional analogs of a MS4A polypeptide. Functional analogs share at least one biological function with a MS4A polypeptide. An exemplary function is immunogenicity. In the context of amino acid sequence, biologically functional analogs, as used herein, are peptides in which certain, but not most or all, of the amino acids can be substituted. Functional analogs can be created at the level of the corresponding nucleic acid molecule, altering such sequence to encode desired amino acid changes. In one embodiment, changes can be introduced to improve the antigenicity of the protein. In another embodiment, a MS4A polypeptide sequence is varied so as to assess the activity of a mutant MS4A polypeptide.

The present invention also encompasses recombinant production of the disclosed MS4A polypeptides. Briefly, a nucleic acid sequence encoding a MS4A polypeptide, or portion thereof, is cloned into a expression cassette, the cassette is introduced into a host organism, where it is recombinantly produced.

The term “expression cassette” as used herein means a DNA sequence capable of directing expression of a particular nucleotide sequence in an appropriate host cell, comprising a promoter operably linked to the nucleotide sequence of interest which is operably linked to termination signals. It also typically comprises sequences required for proper translation of the nucleotide sequence. The expression cassette comprising the nucleotide sequence of interest can be chimeric. The expression cassette can also be one which is naturally occurring but has been obtained in a recombinant form useful for heterologous expression.

The expression of the nucleotide sequence in the expression cassette can be under the control of a constitutive promoter or an inducible promoter which initiates transcription only when the host cell is exposed to some

particular external stimulus. Exemplary promoters include Simian virus 40 early promoter, a long terminal repeat promoter from retrovirus, an action promoter, a heat shock promoter, and a metallothien protein. In the case of a multicellular organism, the promoter and promoter region can direct expression to a particular tissue or organ or stage of development. Exemplary tissue-specific promoter regions include a MS4A promoter, described herein. Suitable expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus, yeast vectors, bacteriophage vectors (e.g., lambda phage), and plasmid and cosmids DNA vectors.

The term “host cell”, as used herein, refers to a cell into which a heterologous nucleic acid molecule has been introduced. Transformed cells, tissues, or organisms are understood to encompass not only the end product of a transformation process, but also transgenic progeny thereof.

A host cell strain can be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. For example, different host cells have characteristic and specific mechanisms for the translational and post-transactional processing and modification (e.g., glycosylation, phosphorylation of proteins). Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. Expression in a bacterial system can be used to produce a non-glycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in animal cells can be used to ensure "native" glycosylation of a heterologous protein.

Expression constructs are transfected into a host cell by any standard method, including electroporation, calcium phosphate precipitation, DEAE-Dextran transfection, liposome-mediated transfection, and infection using a retrovirus. The MS4A-encoding nucleotide sequence carried in the expression construct can be stably integrated into the genome of the host or it can be present as an extrachromosomal molecule.

Isolated polypeptides and recombinantly produced polypeptides can be purified and characterized using a variety of standard techniques that are well known to the skilled artisan. See, *e.g.* Ausubel et al. (1992), Bodanszky, et al. (1976) Peptide Synthesis, John Wiley and Sons, Second Edition, New York, New York and Zimmer et al. (1993) Peptides, pp. 393–394, ESCOM Science Publishers, B. V.

I.C. Nucleotide and Amino Acid Sequence Comparisons

The terms "identical" or percent "identity" in the context of two or more nucleotide or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms disclosed herein or by visual inspection.

The term "substantially identical" in regards to a nucleotide or polypeptide sequence means that a particular sequence varies from the sequence of a naturally occurring sequence by one or more deletions, substitutions, or additions, the net effect of which is to retain at least some of biological activity of the natural gene, gene product, or sequence. Such sequences include "mutant" sequences, or sequences wherein the biological activity is altered to some degree but retains at least some of the original biological activity. The term "naturally occurring", as used herein, is used to describe a composition that can be found in nature as distinct from being artificially produced by man. For example, a protein or nucleotide sequence present in an organism, which can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory, is naturally occurring.

For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer program, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are selected. The sequence comparison algorithm then calculates the percent sequence

identity for the designated test sequence(s) relative to the reference sequence, based on the selected program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman (1981) *Adv Appl Math* 2:482, by the homology alignment algorithm of Needleman & Wunsch (1970) *J Mol Biol* 48:443, by the search for similarity method of Pearson & Lipman (1988) *Proc Natl Acad Sci USA* 85:2444-2448, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, WI), or by visual inspection. <u>See</u> *generally,* Ausubel et al., 1992.

A preferred algorithm for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al. (1990) *J Mol Biol* 215: 403-410. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold. These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when the cumulative alignment score falls off by the quantity X from its maximum achieved value, the cumulative score goes to zero or below due to the accumulation of one or more negative-scoring residue alignments, or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the

alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength W=11, an expectation E=10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix. See Henikoff & Henikoff (1989) *Proc Natl Acad Sci USA* 89:10915.

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences. See, *e.g.*, Karlin and Altschul (1993) *Proc Natl Acad Sci USA* 90:5873-5887. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a test nucleic acid sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid sequence to the reference nucleic acid sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

I.D. Antibodies

The present invention also provides an antibody that specifically binds a MS4A polypeptide. The term “antibody” indicates an immunoglobulin protein, or functional portion thereof, including a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a single chain antibody, Fab fragments, and an Fab expression library. “Functional portion” refers to the part of the protein that binds a molecule of interest. In a preferred embodiment, an antibody of the invention is a monoclonal antibody. Techniques for preparing and characterizing antibodies are well known in the art (See, *e.g.*, Harlow & Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). A monoclonal antibody of the present invention can be readily prepared through use of well-known techniques such as the hybridoma techniques exemplified in U.S. Patent No 4,196,265 and the phage-displayed techniques disclosed in U.S. Patent No. 5,260,203.

The phrase "specifically (or selectively) binds to an antibody", or "specifically (or selectively) immunoreactive with", when referring to a protein or peptide, refers to a binding reaction which is determinative of the presence of the protein in a heterogeneous population of proteins and other biological materials. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein and do not show significant binding to other proteins present in the sample. Specific binding to an antibody under such conditions can require an antibody that is selected for its specificity for a particular protein. For example, antibodies raised to a protein with an amino acid sequence encoded by any of the nucleic acid sequences of the invention can be selected to obtain antibodies specifically immunoreactive with that protein and not with unrelated proteins.

The use of a molecular cloning approach to generate antibodies, particularly monoclonal antibodies, and more particularly single chain monoclonal antibodies, are also provided. The production of single chain antibodies has been described in the art. See, *e.g.*, U.S. Patent No. 5,260,203. For this approach, combinatorial immunoglobulin phagemid libraries are prepared from RNA isolated from the spleen of the immunized animal, and phagemids expressing appropriate antibodies are selected by panning on endothelial tissue. The advantages of this approach over conventional hybridoma techniques are that approximately 10^4 times as many antibodies can be produced and screened in a single round, and that new specificities are generated by heavy (H) and light (L) chain combinations in a single chain, which further increases the chance of finding appropriate antibodies. Thus, an antibody of the present invention, or a "derivative" of an antibody of the present invention, pertains to a single polypeptide chain binding molecule which has binding specificity and affinity substantially similar to the binding specificity and affinity of the light and heavy chain aggregate variable region of an antibody described herein.

The term "immunochemical reaction", as used herein, refers to any of a variety of immunoassay formats used to detect antibodies specifically bound to a particular protein, including but not limited to competitive and

non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, *in situ* immunoassays (*e.g.*, using colloidal gold, enzyme or radioisotope labels), western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. See Harlow & Lane (1988) for a description of immunoassay formats and conditions.

I.E. Protein Binding Assays

The term “binding” refers to an affinity between two molecules, for example, a ligand and a receptor. As used herein, “binding” means a preferential binding of one molecule for another in a mixture of molecules. The binding of the molecules can be considered specific if the binding affinity is about 1 x 10^4 M^{-1} to about 1 x 10^6 M^{-1} or greater. Binding of two molecules also encompasses a quality or state of mutual action such that an activity of one protein or compound on another protein is inhibitory (in the case of an antagonist) or enhancing (in the case of an agonist). Exemplary protein binding assays include but are not limited to Fluorescence Correlation Spectroscopy (FCS), Surface-Enhanced Laser Desorption/Ionization time-of-flight mass spectrometry (SELDI-TOF), and Biacore, each described further herein below.

Fluorescence Correlation Spectroscopy (FCS) measures the average diffusion rate of a fluorescent molecule within a small sample volume (Madge et al. (1972) *Phys Rev Lett* 29:705-708; Maiti et al. (1997) *Proc Natl Acad Sci USA*, 94:11753-11757). The sample size can be as low as 10^3 fluorescent molecules and the sample volume as low as the cytoplasm of a single bacterium. The diffusion rate is a function of the mass of the molecule and decreases as the mass increases. FCS can therefore be applied to protein-ligand interaction analysis by measuring the change in mass and therefore in diffusion rate of a molecule upon binding. In a typical

experiment, the target to be analyzed is expressed as a recombinant protein with a sequence tag, such as a poly-histidine sequence, inserted at the N-terminus or C-terminus. The expression takes place in *E. coli*, yeast or mammalian cells. The protein is purified using chromatographic methods. For example, the poly-histidine tag can be used to bind the expressed protein to a metal chelate column such as Ni^{2+} chelated on iminodiacetic acid agarose. The protein is then labeled with a fluorescent tag such as carboxytetramethylrhodamine or BODIPY™ (Molecular Probes, Eugene, Oregon). The protein is then exposed in solution to the potential ligand, and its diffusion rate is determined by FCS using instrumentation available from Carl Zeiss, Inc. (Thornwood, New York). Ligand binding is determined by changes in the diffusion rate of the protein.

Surface-Enhanced Laser Desorption/Ionization (SELDI) was developed by Hutchens & Yip (1993) *Rapid Commun Mass Spectrom* 7:576-580). When coupled to a time-of-flight mass spectrometer (TOF), SELDI provides a means to rapidly analyze molecules retained on a chip. It can be applied to ligand-protein interaction analysis by covalently binding the target protein on the chip and analyzing by MS the small molecules that bind to this protein (Worrall et al. (1998) *Anal Biochem* 70:750-756). In a typical experiment, the target to be analyzed is expressed as described for FCS. The purified protein is then used in the assay without further preparation. It is bound to the SELDI chip either by utilizing the poly-histidine tag or by other interaction such as ion exchange or hydrophobic interaction. The chip thus prepared is then exposed to the potential ligand via, for example, a delivery system able to pipet the ligands in a sequential manner (autosampler). The chip is then washed in solutions of increasing stringency, for example a series of washes with buffer solutions containing an increasing ionic strength. After each wash, the bound material is analyzed by submitting the chip to SELDI-TOF. Ligands that specifically bind the target are identified by the stringency of the wash needed to elute them.

Biacore relies on changes in the refractive index at the surface layer upon binding of a ligand to a protein immobilized on the layer. In this system, a collection of small ligands is injected sequentially in a 2-5 microliter cell, wherein the protein is immobilized within the cell. Binding is detected by surface plasmon resonance (SPR) by recording laser light refracting from the surface. In general, the refractive index change for a given change of mass concentration at the surface layer is practically the same for all proteins and peptides, allowing a single method to be applicable for any protein (Liedberg et al. (1983) *Sensors Actuators* 4:299-304; Malmquist (1993) *Nature* 361:186-187). In a typical experiment, the target to be analyzed is expressed as described for FCS. The purified protein is then used in the assay without further preparation. It is bound to the Biacore chip either by utilizing the poly-histidine tag or by other interaction such as ion exchange or hydrophobic interaction. The chip thus prepared is then exposed to the potential ligand via the delivery system incorporated in the instruments sold by Biacore (Uppsala, Sweden) to pipet the ligands in a sequential manner (autosampler). The SPR signal on the chip is recorded and changes in the refractive index indicate an interaction between the immobilized target and the ligand. Analysis of the signal kinetics of on rate and off rate allows the discrimination between non-specific and specific interaction.

I.F. Transgenic animals

It is also within the scope of the present invention to prepare a transgenic animal to mutagenize the MS4A locus or to express a transgene comprising nucleic acid sequences of the present invention. The term "transgenic animal", indicates an animal comprising a germline insertion of a heterologous nucleic acid. Transgenic animals of the present invention are understood to encompass not only the end product of a transformation method, but also transgenic progeny thereof.

The term "transgene", as used herein indicates a heterologous nucleic acid molecule that has been transformed into a host cell. For intended use in the creation of a transgenic animal, the transgene includes

genomic sequences of the host organism at a selected locus or site of transgene integration to mediate a homologous recombination event. A transgene further comprises nucleic acid sequences of interest, for example a targeted modification of the gene residing within the locus, a reporter gene, or a expression cassette, each defined herein above.

Transgene integration can be used to create gene mutations, including "knock-out", "knock-in", or a "knock-down" mutations. Representative approaches are disclosed in the Examples presented below. The term "knock-out" refers to a homologous recombination event that renders a gene inactive. Gene knock-out is generally accomplished by integration of the transgene at a chromosomal loci, thereby interrupting a gene residing at that loci. The term "knock-in" refers to *in vivo* replacement at a targeted locus. Knock-in mutations can modify a gene sequence to create a loss-of-function or gain-of-function mutation. The term "gene knock-down" refers to a homologous recombination event wherein the transgene partially eliminates gene function. A knock-down animal can be created by transgenic expression of an antisense molecule, wherein a transgene comprising the antisense sequence and a relevant promoter are integrated into the genome at a non-essential loci. Expression of the antisense or ribozyme molecule disrupts the corresponding gene function, although this disruption is generally incomplete (Luyckx et al. (1999) *Proc Natl Acad Sci U S A* 96(21):12174-12179).

Conditional mutation can be accomplished using transgenic methods in combination with the Cre-recombinase system in mice. Briefly, in one instance, a transgenic mouse is derived that expresses Cre-recombinase under the direction of an inducible promoter. A second transgenic mouse bears a mutation of a gene of interest as well as a lox-P-flanked endogenous gene sequence. Such transgenic mice are mated, the resulting progeny having both the Cre-recombinase and lox-P-flanked transgenes. Induction of Cre recombinase catalyzes excision of the lox-P-flanked transgene, thereby excising a portion of the endogenous gene sequence and revealing the mutated sequence. Conditional knockout can be varied according to the

temporal and spatial features of Cre recombinase expression, inherent in the selection of a promoter to drive Cre recombinase. See Postic et al. (1999) *J Biol Chem* 275(1):305-315; and Sauer (1998) *Methods* 14(4):381-392.

Transgenes can also be used for heterologous expression in a host organism without generating phenotypically apparent mutations. By this method, nucleotide sequences of interest are introduced into the genome at a nonessential loci, whereby insertion alone does not disrupt an essential gene function. Optionally, expression of the transgene can generate a gain-of-function or ectopic function phenotype.

Techniques for the preparation of transgenic animals are known in the art. Exemplary techniques are described in U.S. Patent No. 5,489,742 (transgenic rats); U.S. Patent Nos. 4,736,866, 5,550,316, 5,614,396, 5,625,125 and 5,648,061 (transgenic mice); U.S. Patent No. 5,573,933 (transgenic pigs); 5,162,215 (transgenic avian species) and U.S. Patent No. 5,741,957 (transgenic bovine species). Briefly, nucleotide sequences of interest are cloned into a vector, and the construct is transformed into a germ cell. In the germ cell, a chromosomal rearrangement event takes place wherein the nucleic acid sequences of interest are integrated into the genome of the germ cell by homologous recombination. Fertilization and propagation of the transformed germ cell results in a transgenic animal. Homozygosity of the mutation is accomplished by intercrossing.

I.G. Therapeutic Methods

The present invention further provides methods for discovering substances that can be used as pharmaceutical compositions. The term "pharmaceutical composition" or "drug" as used herein, each refer to any substance having a biological activity. Substances discovered by methods of the present invention include but are not limited to polypeptide, proteins, peptides, chemical compounds, and antibodies.

A composition of the present invention is typically formulated using acceptable vehicles, adjuvants, and carriers as desired.

Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, and isotonic sodium chloride solution. In

addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectable compositions.

Injectable preparations, for example sterile injectable aqueous or oleaginous suspensions, are formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic diluent or solvent, for example 1,3-butanediol.

A vector can be used as a carrier, for example an adenovirus vector, can be used for gene therapy methods. The vector is purified to sufficiently render it essentially free of undesirable contaminants, such as defective interfering adenovirus particles or endotoxins and other pyrogens such that it does not cause any untoward reactions in the individual receiving the vector construct. A preferred means of purifying the vector involves the use of buoyant density gradients, such as cesium chloride gradient centrifugation.

A transfected cell can also serve as a carrier. By way of example, a liver cell can be removed from an organism, transfected with a nucleic acid sequence of the present invention using methods set forth above and then the transfected cell returned to the organism (*e.g.* injected intra-vascularly).

Monoclonal antibodies or polypeptides of the invention can be administered parenterally by injection or by gradual infusion over time. Although the tissue to be treated can typically be accessed in the body by systemic administration and therefore most often treated by intravenous administration of therapeutic compositions, other tissues and delivery means are provided where there is a likelihood that the tissue targeted contains the target molecule and are known to those of skill in the art.

Representative antibodies for use in the present invention are intact immunoglobulin molecules, substantially intact immunoglobulin molecules, single chain immunoglobulins or antibodies, those portions of an immunoglobulin molecule that contain the paratope, including antibody

fragments. It is within the scope of the present invention that a monovalent modulator can optionally be used.

Methods of preparing "humanized" antibodies are generally well known in the art, and can readily be applied to the antibodies of the present invention. Humanized monoclonal antibodies offer particular advantages over monoclonal antibodies derived from other mammals, particularly insofar as they can be used therapeutically in humans. Specifically, humanized antibodies are not cleared from the circulation as rapidly as "foreign" antigens, and do not activate the immune system in the same manner as foreign antigens and foreign antibodies.

With respect to the therapeutic methods of the present invention, a preferred subject is a vertebrate subject. A preferred vertebrate is warm-blooded; a preferred warm-blooded vertebrate is a mammal. A preferred mammal is a mouse or, most preferably, a human. As used herein and in the claims, the term “patient” includes both human and animal patients. Thus, veterinary therapeutic uses are provided in accordance with the present invention.

Also provided is the treatment of mammals such as humans, as well as those mammals of importance due to being endangered, such as Siberian tigers; of economical importance, such as animals raised on farms for consumption by humans; and/or animals of social importance to humans, such as animals kept as pets or in zoos. Examples of such animals include but are not limited to: carnivores such as cats and dogs; swine, including pigs, hogs, and wild boars; ruminants and/or ungulates such as cattle, oxen, sheep, giraffes, deer, goats, bison, and camels; and horses. Also provided is the treatment of birds, including the treatment of those kinds of birds that are endangered and/or kept in zoos, as well as fowl, and more particularly domesticated fowl, *i.e.*, poultry, such as turkeys, chickens, ducks, geese, guinea fowl, and the like, as they are also of economical importance to humans. Thus, provided is the treatment of livestock, including, but not limited to, domesticated swine, ruminants, ungulates, horses, poultry, and the like.

As used herein, the term “experimental subject” refers to any subject or sample in which the desired measurement is unknown. The term “control subject” refers to any subject or sample in which a desired measure is unknown.

As used herein, an “effective” dose refers to a dose(s) administered to an individual patient sufficient to cause a change in MS4A activity. One of ordinary skill in the art can tailor the dosages to an individual patient, taking into account the particular formulation and method of administration to be used with the composition as well as patient height, weight, severity of symptoms, and stage of the biological condition to be treated. Such adjustments or variations, as well as evaluation of when and how to make such adjustments or variations, are well known to those of ordinary skill in the art of medicine.

A therapeutically effective amount can comprise a range of amounts. One skilled in the art can readily assess the potency and efficacy of a MS4A modulator of this invention and adjust the therapeutic regimen accordingly. A modulator of MS4A biological activity can be evaluated by a variety of means including the use of a responsive reporter gene, interaction of MS4A polypeptides with a monoclonal antibody, analysis of cell subpopulations, and measurement of $[Ca^{2+}]_i$ levels, each technique described herein.

Additional formulation and dose techniques have been described in the art, see for example, those described in U.S. Patent Nos. 5,326,902 and 5,234,933, and International Publication No. WO 93/25521.

For the purposes described above, the identified substances can normally be administered systemically, parenterally, or orally. The term “parenteral” as used herein includes intravenous, intra-muscular, intra-arterial injection, or infusion techniques. Other compositions for administration include liquids for external use, and endermic liniments (ointment, etc.), suppositories, and pessaries which comprise one or more of the active substance(s) and can be prepared by known methods.

II. CD20 Gene Family Members

II.A. Identification of CD20 Gene Family Members

The present invention provides MS4A nucleic acid and polypeptide sequences. Preferably, a MS4A gene comprises the sequence set forth as any one of the odd-numbered SEQ ID NOs:1-37, a nucleic acid molecule that is substantially similar to any one of the odd-numbered SEQ ID NOs:1-37, or a nucleic acid molecule comprising a 20 base pair nucleotide sequence that is identical to a contiguous 20 base pair sequence of any one of the odd-numbered SEQ ID NOs:1-37.

To identify new CD20 gene family members, the human and mouse CD20 amino acid sequences (Tedder et al., 1988a; Tedder et al., 1988b) were used to search the translated GenBank databases, including expressed sequence tags, using the BLAST program (Altschul et al., 1997). Among 337 homologous sequences identified, at least 17 novel genes expressed by mouse, human, and pig had predicted amino acid sequences homologous to CD20. Complete coding regions were predicted using overlapping nucleotide sequences obtained from sequenced ESTs and cDNAs that corresponded to unique, near full-length transcripts in humans and mice (Figure 1). All nucleotide sequences were verified by sequencing multiple near full-length cDNAs isolated by applicants and 40 cDNAs obtained from the ATCC (American Tissue Culture Collection, Bethesda, Maryland, USA). In addition, a pig cDNA and its human counterpart homologous to CD20 were identified as GenBank submissions AJ236932.1 and AK000224, respectively. In total, unique cDNA clones were identified that encode at least 16 distinct full-length CD20-like proteins.

Complete cDNA sequences encoding the human and mouse MS4A family members (*MS4A1, -A2, -A3, -A4A, -A5, -A6A, -A7, -A8B* and *-A12*) were also used to search the GenBank human genomic database (htgs; http://www.ncbi.nlm.nih.gov/blast/) using the BLAST program (Altschul et al., 1997), as further described in Example 2. Two-hundred-twenty different contigs or distinct genomic DNA sequences were identified in the database of unfinished human genomic sequences that were either identical or similar

to MS4A family members. These sequences were predominantly derived from sixteen partially sequenced bacterial artificial chromosomes (BACs) that spanned 400-500 kb of human chromosome 11q12 (Table 1). Based on known cDNA sequences of MS4A family members, we were able to order and arrange these genomic sequences into overlapping continuous DNA segments. Since many of the contigs identified were overlapping, it was thereby possible to assemble long DNA sequences that encoded entire MS4A genes or portions of MS4A genes. Gaps between exon encoding DNA sequences were filled in many cases by additional sequence homology searches using DNA sequences found at the ends of gaps. When sequence differences were observed between different overlapping DNA fragments, consensus sequences were used or PCR primers were generated, that portion of genomic DNA was then amplified and sequenced to resolve ambiguous sequences.

BLAST analysis of the htgs phase 1 or phase 2 human genomic DNA sequences encoding MS4A cDNAs and the assembled and annotated human genomic sequence thereof, as disclosed herein, revealed the presence of each known human MS4A family member. In addition, three putative genes encoding unique MS4A family members were identified that localized to the q12-13.1 region of human chromosome 11. Complete coding regions were predicted using overlapping nucleotide sequences obtained from sequenced ESTs and cDNAs and by comparison of gene structure, described further herein below (Figure 2).

By identifying sequences that correlated with different MS4A genes in each BAC (Table 1), and by the assembly of minimal genomic DNA lengths that could encode each *MS4A* gene (Figure 2), we used the overlapping BACs to identify the order of the MS4A genes on chromosome 11q12 (Figure 6). This analysis also allowed us to determine the direction of gene transcription for most MS4A genes. Furthermore, the MS4A cDNA sequences, disclosed herein, were used to assemble genomic clones set forth as SEQ ID NOs:73-81. In some cases, multiple MS4A genes could be aligned within a continuous genomic sequence. For example, the genomic

sequence set forth as SEQ ID NO:77 comprises both the *MS4A4E* and *MS4A6A* genes. Similarly, the genomic region set forth as SEQ ID NO:79 comprises three MS4A genes: *MS4A7*, *MS4A5*, and *MS4A12*.

The *MS4A4E* gene encodes 660 bp of translated sequence (Figure 3), contained within at least seven exons (Figure 2). Exons were identified based on their sequence similarities with *MS4A4A* sequences and the identification of canonical splice-donor and -acceptor sites (Aebi & Weissmann, 1987). The *MS4A4E* gene sequence was at least 23,379 base pairs in length, if counted from the putative translation initiation ATG site until the TGA translation termination stop site (Figure 2). An exon encoding the putative 5' untranslated region of *MS4A4E*, was highly homologous with the corresponding sequence in *MS4A4A* cDNAs (disclosed herein). This sequence homology extended for >7 kbp upstream from this putative exon and also included upstream repetitive Alu elements. Representative upstream homologous sequences are shown in Figure 3. Similar sequence homologies were identified in the 3' untranslated regions of *MS4A4E* and *MS4A4A*, which extended beyond the poly-A attachment signal sequences (Figure 3). Based on the sequence similarities in translated and untranslated exons, it appears that the *MS4A4E* and *MS4A4A* genes resulted from a recent gene duplication event.

The *MS4A6E* gene encodes 441 bp of translated sequence (Figure 4), contained within at least four exons (Figure 2). Exons were identified based on their sequence similarities with *MS4A6A* cDNA sequences and the identification of canonical splice-donor and -acceptor sites (Aebi & Weissmann, 1987). In addition, the predicted gene sequences matched those found in three cDNA clones that were sequenced (ATCC Nos. 3704466, 1852248 and 3557769). The *MS4A6E* gene was at least 5,060 bp in length, if counted from the putative translation initiation ATG site until the TGA translation termination codon (Figure 2). The *MS4A6E* gene lacks exons that encode the first two membrane spanning domains present in most MS4A family proteins (Figures 2 and 7). An exon homologous with the 5' untranslated region of *MS4A6A* cDNAs was not identified within 7,629 bp

of sequence upstream of the exon encoding the translation initiation site of *MS4A6E*. However, there was a canonical 3' splice region upstream of the ATG initiation codon located at identical positions in the *MS4A6E* and *MS4A6A* genes. Similar sequence homologies were identified in the 3' untranslated regions of *MS4A6E* and *MS4A6A* that extend beyond the sequence shown in Figure 4. Based on the sequence similarities in translated and untranslated exons, it appears that the *MS4A6E* and *MS4A6A* genes represent a recent gene duplication event, although several exons encoding translated sequence were lost in the *MS4A6E* gene (Figure 2).

The *MS4A10* gene encodes 726 bp of translated sequence (Figure 5), contained within at least six exons (Figure 2). Exons were identified based on their sequence similarities with mouse *MS4a10* cDNA sequences and the identification of canonical splice-donor and -acceptor sites (Aebi & Weissmann, 1987). The *MS4A10* gene was at least 8,183 bp in length if counted from the putative translation initiation ATG site until the TGA translation termination stop site (Figure 2). An exon homologous with the 5' untranslated region of mouse *MS4a10* cDNAs was not identified within 8,829 bp of sequence upstream of the exon encoding the translation initiation site of *MS4A10*. However, there was a canonical 3' splice region upstream of the ATG initiation codon located at identical positions in the *MS4A10* and *MS4a10* genes. Modest sequence homologies were identified in the 3' untranslated regions of *MS4A10* and *MS4a10* (Figure 5).

Table 1

Human BACs Containing MS4A Genes

BAC	Accession No.[a]	Chromosome	MS4A gene[b]
RP11-206B10	AC009703	15	A4A, A4E, A6A
RP11-21B14	AC013807	unknown	A6A, A2, A3
RP11-24D1	AC015840	unknown	A4A, A5, A6E, A7
RP11-652L5	AC018966	11	A4A, A4E, A6A
RP11-448N3	AC024066	11	A8B
RP11-312N17	AC027599	11	A8B, A10
RP11-196E16	AC027787	15	A5, A1
CMB9-79B2	AP000748	11q23	A10
RP11-804A23	AP000777	11	A10
RP11-736I10	AP000790	11q12	A3
RP11-804B24	AP000934	11	A10
RP11-729B4	AP001034	11q12	A5, A12, A1
CMB9-2M23	AP001181	11q12	A2, A3
CMB9-100I1	AP001257	11q12	A6A, A4E
CMB9-49F18	AP001259	11	A8B
RP11-68H20	AP001986	11q	A10

[a]GenBank Accession number for the indicated BAC.

[b]indicates the MS4A gene sequences that mapped to each BAC.

II.B. MS4A Nomenclature

In collaboration with the Human Gene Nomenclature Committee (www.gene.ucl.ac.uk/nomenclature/), this gene family was designated as the MS4A family (Membrane Spanning 4-domain family, subfamily A). The MS4 designation is to accommodate the future identification of genes encoding proteins with a similar structure, yet with unresolved functions. Subfamily A will designate the *CD20* family. Using this nomenclature, the *CD20* gene was designated as *MS4A1*, *FcεRIβ* as *MS4A2*, and HTm4 as *MS4A3*. Among the 16 novel genes identified, 8 human genes were named *MS4A4A*, *MS4A4E, MS4A5*, *MS4A6A*, *MS4A6E*, *MS4A7*, *MS4A8B,* and *MS4A12*. A ninth gene encoded a protein homologous with the single member of the mouse MS4a10 subfamily. This gene was tentatively designated as *MS4A10*. The remaining genes were of mouse or pig origin and were therefore labeled as *MS4a3-MS4a12* based on the nomenclature of homologous genes corresponding to human counterparts. Distinct mouse genes that encoded proteins with highly homologous sequences were designated as *MS4a4B, MS4a4C, MS4a4D, and as MS4a6B, MS4a6C, and MS4a6D* to signify close homology.

II.C. MS4A Gene Chromosome Locations

Chromosome locations for the human *MS4A4A, MS4A6A*, *MS4A7,* and *MS4A8B* genes were identified in two distinct homology searches. Regions of human *MS4A4A* (bp 1286-1588), *MS4A6A* (bp 682-1106), *MS4A7* (bp 502-941), *MS4A7* (bp 1015-1177), and *MS4A8B* (bp 1007-1350), were 98%, 98%, 97%, 99% and 97% identical with human STS genomic sequence tag sites, WI-11578, SHGC-36634, WI-12101, WIAF-3856, and WI-14145, respectively (http://www.ncbi.nlm.nih.gov/blast). These genomic sequence tag sites are located on human chromosome 11 at Genomic Database locus D11S1357-D11S913, which maps to 11q12-13 (http://www.ncbi.nlm.nih.gov/genemap). These mapping results were confirmed using the UniGene collection at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/Genemap98/) for expressed sequence tags identical to human *MS4A4A*, *MS4A6A*, *MS4A7*,

MS4A8B sequences. By this analysis, at least 7 of the 9 currently identified human MS4A genes are clustered.

The organization of the 12 MS4A genes on human chromosome 11 was determined by identifying sequenced human genomic DNA fragments (contigs of different lengths) from 15 BAC clones (Table 1). Contiguous DNA segments for each BAC were constructed based on human MS4A exon and cDNA sequences, and overlapping contigs. Although some gaps were present in MS4A gene introns (Figure 2) or between MS4A genes, the relative position of each gene on chromosome 11q12-13.1 was determined (Figure 6). *MS4A1* was located in a telemetric region of 11q12-13.1 compared with *MS4A2* and *MS4A3*. Seven *MS4A* genes were located in between *MS4A1* and *MS4A2*. Two other *MS4A* genes, *MS4A8B* and *MS4A10* were centromeric to *MS4A2* and *MS4A3*, although the distance between these genes was not determined. Interestingly, *MS4A6A*, *MS4A4E*, *MS4A4A* and *MS4A6E* were arranged linearly suggesting that these genes might have arisen through the duplication of a single genomic element. It is envisioned that this genetic locus extends further and contains additional MS4A genes.

<u>II.A.</u> <u>MS4A Gene Structure</u>

Complete coding region sequences were verified for each deduced protein, except for the *MS4a3* cDNA that was not full-length (Figure 1). Proposed ATG translation initiation codons were based on the translation initiation consensus sequence, ANNATG (Kozak (1986) *Cell* 44:283-292), and the existence of in-frame upstream translation stop codons in most cases. Whether the first or second ATG codon in mouse *MS4a8B* was used for translation initiation was unknown although the second ATG was identical with the start codon of human *MS4A8B* (Figure 7).

Poly(A) attachment signal sequences were identified in the proximal 3' untranslated regions of each gene product except *MS4A6A*, *MS4A6E*, *MS4A10*, and *MS4a6C*. Two poly(A) signal sequences were found in *MS4a4D, MS4A5,* and *MS4a10* transcripts, while four were observed in *MS4A4A* transcripts.

The disclosed MS4A cDNAs were further used to annotate the genomic sequence derived from BAC clones. Annotated features include definition of coding regions, intron|exon junctions, sequences upstream of the initial coding region of each gene that comprise the promoter region, and other adjacent sequences that could also comprise gene regulatory elements. Representative methods for further characterizing a MS4A promoter region are disclosed in Example 9.

Annotation of human MS4A genomic regions (SEQ ID NOs:73-81), as disclosed herein, enabled a comparison of gene structure among MS4A genes. The overall domain organization of each MS4A gene was similar (Figures 2 and 7). All exon|intron|exon boundaries were consistent with consensus splice-donor and -acceptor sequences unless otherwise indicated, with exon|GTGAGT-intron-CAG|exon sequences in most cases (Aebi & Weissmann, 1987). In addition, the splice junctions for all translated exons were located after the third nucleotide in each codon. Most MS4A proteins were encoded by 6 exons except MS4A2, MS4A5, and MS4A6E (Figure 2 and 7). In these exceptions: the N-terminal cytoplasmic domain of MS4A2 was encoded by two exons (Küster et al., 1992); the *MS4A5* and *MS4A6E* genes did not encode C-terminal cytoplasmic domains; and the *MS4A6E* gene had only two membrane spanning domains. Intron lengths demonstrated wide variation from 181 bp in *MS4A12* to 13,731 bp in *MS4A5*. In some cases however, exact intron lengths were not determined; *MS4A3*, *MS4A4*, and *MS4A12* (Figure 2). Distances between translation initiation and termination codons were determined for most MS4A genes; with *MS4A6E* being the smallest (5,060 bp) and *MS4A4E* being the longest (23,379 bp) genes (Figure 6). Thus, the intron|exon organization of all MS4A family members is consistent with the high degree of conservation within this gene family.

There were no amino-terminal signal sequences, although all MS4A proteins contained hydrophobic regions of sufficient length to pass through the membrane at least four times. Notable was a marked clustering of charged residues at both ends of the putative transmembrane domains,

some of which were highly conserved. In some cases, the first and second putative transmembrane domains of MS4A proteins were a continuous stretch of hydrophobic amino acids without an obvious inter-transmembrane hydrophilic bridge. By contrast, MS4A4A and MS4A7 had 6 to 7 hydrophilic amino acids inserted between the first and second hydrophobic domains. In human MS4A4A and mouse MS4a4B, MS4a4C, and MS4a4D, an extensive hydrophobic region followed the fourth putative membrane-spanning domain. Thus, the overall structure of MS4A family members was well conserved.

II.E. MS4A Gene Splice Variants

Among the MS4A cDNAs sequenced and EST sequences analyzed, multiple splice variants were identified that encoded variant MS4A proteins. In most cases, exons were spliced out, which generated truncated protein products. Potential splice variants of the *MS4A4A*, *MS4A5*, *MS4A6A*, and *MS4A7* genes were identified. Whether these alternatively spliced variants produce functional proteins has yet to be determined.

Two splice variations of the *MS4A4A* gene were identified during an analysis of *MS4A4A* mRNA expression by lymphoblastoid cell lines. Most of the hematopoietic cell lines examined expressed transcripts encoding a full-length MS4A4A protein as shown in Figure 7. However, a second smaller transcript was also expressed in most cases that contained a potential exon deletion of 158 nucleotides. This was a frequent event since 40% of *MS4A4A* cDNAs generated from the BJAB B cell line encoded the truncated protein. In addition, the same splicing event was observed in two of five EST sequences that covered this region of the MS4A4A protein. Splicing-out this potential exon deleted the third membrane-spanning domain and the second extracellular loop from the full-length protein (positions 110-163, Figure 3). Of interest, this splicing event fused the first/second membrane spanning domains with the fourth membrane spanning domain. However, the fourth transmembrane spanning domain in MS4A4A is followed by another hydrophobic region of sufficient length to traverse the membrane (disclosed herein). This suggests that differential splicing can generate an

alternative MS4A4A protein with four membrane spanning domains lacking a significant extracellular domain.

In the case of the *MS4A5* gene, two of nine *MS4A5* EST sequences analyzed (GenBank Accession Nos. AA411806 and AA781801) encoded a splice variant that preserved the reading frame of the transcript. In both sequences, the exon encoding the third membrane-spanning domain and the second extracellular loop from the full-length protein (TM3, Figure 1) was spliced out using normal splice-donor and -acceptor sequences, which deleted 51 amino acids (114-164) from the full length protein (Figure 7). This deletion resulted in a protein with the first/second membrane spanning domains fused with the fourth predicted membrane-spanning domain. Thus, the truncated MS4A5 protein would possess three membrane-spanning domains with an extracellular carboxyl-terminal domain.

A novel splicing event was observed in the *MS4A6A* gene which resulted in a truncated protein. A novel splice donor site (CAG T^{683}|GT GAG T) is located within the exon encoding the TM3/extracellular loop domains (Figure 4). This cryptic splice donor site was spliced with the normal 3' splice acceptor site of the exon encoding the TM4 domain, which thereby deletes nucleotides 684-787 from *MS4A6A* transcripts (Figure 4). Since there was an extra T introduced into the codon sequence due to this alternative splicing event, there was a frameshift in the coding sequence. This potentially results in the attachment of a novel 30 amino acid sequence (-WNSLSDADLHSAGILPSCAHCCAAVETGLL) that is not predicted to be hydrophobic. Thus, the variant MS4A protein would be 70 amino acids shorter and would lack the fourth membrane-spanning and cytoplasmic domains. This alternative splicing event was found in 3 of 29 EST sequences that encoded this region (GenBank Accession Nos. AI278475, AA461046, and AA448335) and in one cDNA clone (GenBank Accession No. AB013104).

Splice variation in *MS4A7A* transcripts produces two distinct protein products in addition to the presumably normal protein. In one case, a splice variation in *MS4A7A* transcripts produces a protein product similar in

structure to the MS4A6E protein. The exon encoding the first/second membrane spanning domains (amino acids 50-94, Figure 7) was deleted in 2 of 4 *MS4A7* EST sequences analyzed (GenBank Accession Nos. N42191 and R11179) that cover this region. Thus, the protein product would have a longer N-terminal cytoplasmic domain and only two membrane spanning domains. In the second case, the exon encoding the fourth membrane-spanning domain (amino acids 183-216) was deleted in 2 EST sequences (GenBank Accession Nos. R11180 and AI188478) out of 18 sequences analyzed (Figure 7).

II.F. MS4A Gene Polymorphisms

Putative polymorphisms were identified in the *MS4A6A* gene. Two nucleotide substitutions were found in cDNA clone ATCC No. 499181 and in 13 of 38 EST sequences analyzed (Figure 1). The first substitution was at nucleotide 373 that exchanged a C for a T, which did not alter the amino acid sequence. The second substitution resulted in a Ser in place of Thr at amino acid 185. In addition, a third substitution was found in 4 of the 38 EST sequences analyzed where a Ser was substituted in place of an Ala at amino acid position 183. This substitution was paired with a Ser to Thr substitution at amino acid position 185 in half of the clones analyzed. These differences most likely represent common sequence polymorphisms since they were observed in multiple independent cDNA clones. Based on our genetic DNA analysis, it is unlikely that these differences could represent transcripts from distinct genes that are almost identical in coding sequence.

As with the *MS4A6A* gene (disclosed herein), potential gene polymorphisms were observed in *MS4A6E*. Three cDNA clones representing partial transcripts were sequenced completely on both strands. The predicted *MS4A6E* gene product and one cDNA clone (ATCC No. 3704466) had identical sequences. However, the ATCC No. 3557769 cDNA had a nucleotide substitution at position 314 (Figure 4) that exchanged a T for a C, which did not alter the predicted amino acid sequence. The ATCC No.1852248 cDNA clone had the longest insert that starts at nucleotide position 60 and ended at position 661 as shown in Figure 4. This cDNA had

a substitution at nucleotide 153 that exchanged a G for a T, which resulted in a Phe in place of Val at amino acid 47 (Figure 4). Therefore, sequence polymorphisms can exist within the *MS4A6E* gene.

Other potential polymorphisms were observed in other MS4A family members based on consistent nucleotide variations found in *MS4A4E* sequences.

The assembly and annotation of genomic sequences comprising MS4A genes in the region of human chromosome 11q12-13.1, disclosed herein for the first time, provide source material for identification of polymorphisms that are linked to MS4A genes. Such polymorphisms can include single nucleotide polymorphisms as disclosed within the *MS4A6A* and *MS4A6E* coding region sequences. In addition, polymorphisms within or genetically linked to MS4A genes can also comprise restriction length polymorphisms (RFLPs) (Lander & Botstein (1989) *Genetics* 121:185-199), short tandem repeat polymorphisms (STRPs), short sequence length polymorphisms (SSLPs) (Dietrich et al. (1996) *Nature* 380:149-152), amplified fragment length polymorphisms (AFLPs) (Latorra et al. (1994) *PCR Methods Appl* 3(6):351-358), and microsatellite markers (Schalkwyk et al. (1999) *Genome Res* 9:878-887). Identification of polymorphisms within an isolated DNA molecule are known to one of skill in the art.

<u>II.G.</u> <u>MS4A Proteins</u>

The MS4A genes encoded proteins of 16-29 kDa (Table 2).

Table 2

MS4A Family Members

Human		Mouse		Human/Mouse
Name	kDa	Name	kDa	Homology
		MS4a3		63% (partial)
MS4A4A	23			
		Ms4a4B	24	41%
		Ms4a4C	24	44%
		Ms4a4D	24	40%
MS4A4E	24			
MS4A5	22			
MS4A6A	27			
		Ms4a6B	27	52%
		Ms4a6C	24	51%
		Ms4a6D	26	53%
MS4A6E	16			
MS4A7	26	MS4a7	26	53%
MS4A8B	26	MS4a8B	29	63%
MS4A10	27	MS4a10	29	52%
MS4A12	26	MS4a12(pig)	26	60%

[a]Predicted molecular weights for the new MS4A family members and the percentage amino acid sequence identity between deduced MS4A and MS4a proteins.

Comparisons between CD20 and the predicted amino acid sequences for human MS4A4A, MS4A5, MS4A6A, MS4A7, MS4A8B, and MS4A12 revealed 23-29% amino acid sequence identity (Figure 7). The highest degree of identity was found in the first three transmembrane domains with multiple regions of conserved amino acids. In particular, the amino acid sequences LGAXQI (SEQ ID NO:57) and LSLG (SEQ ID NO:58) were common within the first transmembrane domain, GYPFWG (SEQ ID NO:60) and FIISGSLS (SEQ ID NO:61) were common in the second domain, and $SLX_2NX_2SX_3AX_2G$ (SEQ ID NO:62) was found in the third transmembrane domain. The first and second transmembrane domains of MS4A8B were 46% identical in amino acid sequence with human CD20, 41% identical with FcεRIβ, and 39% identical with HTm4. The MS4A4A, MS4A5, MS4A6A, and MS4A7 proteins were most homologous in their first and second transmembrane domains with the human FcεRIβ chain, with 37-46% amino acid sequence identity. There was large variation between MS4A proteins in the N– and C-terminal cytoplasmic domains. However, Pro residues were significantly over-represented within the N– and C-terminal cytoplasmic domains of most MS4A family members. There was some sequence identity in the first potential extracellular loop that was ~13 amino acids in length for each protein. By contrast, the second predicted extracellular loop ranged from 10-46 amino acids in length with diverse sequences.

The putative *MS4A4E* gene encodes a 220 amino acid protein of 23.8 kDa with a predicted amino acid sequence that is 76% identical with the MS4A4A protein (Figure 3). Consistent with other MS4A proteins, the most significant homologies between MS4A4E and other MS4A family members were found in the membrane spanning domains (Figure 7). Common amino acid motifs were readily visualized such as KXLGAIQI (SEQ ID NO:57), GYPXWG (SEQ ID NO:60), and SGXLSI (SEQ ID NO:59) in the first and second hydrophobic regions that represent potential transmembrane regions. The intracellular N- and C-terminal domains were highly conserved

between MS4A4E and MS4A4A, but were divergent from other family members.

The putative *MS4A6E* gene encodes a 147 amino acid protein of 15.9 kDa with a predicted amino acid sequence that is 78% identical with the MS4A6A protein (Figure 4). The most significant homologies between MS4A6E and other MS4A family members were found in the membrane spanning domains, although MS4A6E only had two (TM3 and TM4) membrane-spanning domains (Figures 4 and 7). The putative second extracellular loops of MS4A6E and MS4A6A were of identical length (Figure 4). Common amino acid motifs were readily visualized in the hydrophobic regions that represent potential transmembrane regions. The intracellular N-terminal domain was highly conserved between MS4A6E and MS4A6A, but were divergent from other family members. MS4A6E protein also lacks a C-terminal cytoplasmic domain (Figure 4).

The putative *MS4A10* gene encodes a translated 241 amino acid protein of 26.9 kDa with a predicted amino acid sequence that is 52% identical with the mouse MS4a10 protein (Figure 5). The most significant homologies between MS4A10 and MS4a10 were found in the membrane spanning domains and the putative second extracellular loop (Figure 5). Although the N-terminal cytoplasmic domains of MS4A10 and MS4a10 were of similar length, the intracellular N- and C-terminal domains had the lowest sequence homologies among domains. The cytoplasmic C-terminal domain was 28 amino acids shorter in MS4A10 than MS4a10. Nonetheless, based on the sequence similarities of translated regions, it appears that MS4A10 and MS4a10 represent homologous genes that are more similar to one another than other MS4A family members.

Ten novel mouse MS4A proteins were identified that shared 40-63% amino acid sequence identity with their potential human counterparts (Figure 7, Table 2). For comparison, the mouse and human CD20 proteins are 74% identical in amino acid sequence (Tedder et al., 1988a). A single partial cDNA was identified that encoded the mouse homologue for HTm4 (*MS4a3*, Figure 7). The predicted amino terminus of the proposed MS4a3 protein

was 23 amino acids shorter than in the human protein, although their overlapping regions were 63% identical in amino acid sequence. In all cases, the transmembrane domains of the human and mouse MS4A proteins were the most well conserved regions. For example, the human MS4A8B protein was 78% identical in sequence to MS4a8B in the first 3 transmembrane domains and 68% identical in domain 4. Additional MS4A genes are likely to be identified in humans and mice, including the mouse *MS4A5* homologue.

A UPGMA (unweighted pair group method using arithmetic averages) tree showing relatedness of deduced MS4A and MS4a protein sequences is depicted in Figure 8.

III. Methods for Detecting a MS4A Nucleic Acid Molecule

In another aspect of the invention, a method is provided for detecting a nucleic acid molecule that encodes a MS4A polypeptide. According to the method, a biological sample having nucleic acid material is procured and hybridized under stringent hybridization conditions to a MS4A nucleic acid molecule of the present invention. Such hybridization enables a nucleic acid molecule of the biological sample and the MS4A nucleic acid molecule to form a detectable duplex structure. Preferably, the MS4A nucleic acid molecule includes some or all nucleotides of any one of the odd-numbered SEQ ID NOs:1-37. Also preferably, the biological sample comprises human nucleic acid material.

III.A. Expression of MS4A Family Members in Hematopoietic Cells

Since CD20, FcεRIβ, and HTm4 expression are restricted to hematopoietic tissues, MS4A gene transcription was assessed by PCR amplification of cDNA from eleven human hematopoietic cell lines. Like CD20, *MS4A8B* was only expressed by B cell lines (Table 3). *MS4A5* was only expressed by a promonocytic cell line. *MS4A6A* transcripts were expressed by B cell, myelomonocytic, and erythroleukemia cell lines. *MS4A4A* mRNA was expressed by all cell lines examined, although the relative mRNA levels varied significantly. *MS4A7* was expressed in most,

but not all of the cell lines tested. *MS4A12* transcripts were not detected in these cell lines. Thus, most MS4A family members are likely to be expressed in hematopoietic tissues.

ESTs encoding MS4A transcripts were isolated from a variety of different cDNA libraries. *MS4A4A* ESTs were from aorta, brain, breast, heart, kidney, lung, ovary, pancreas, placenta, prostate, stomach, testis, and uterine tissues. *MS4A5* ESTs were only isolated from testis. *MS4A6A* ESTs were from aorta, brain, the central nervous system, colon, gall bladder, heart, kidney, lung, muscle, ovary, pancreas, placenta, prostate, skin, stomach, tonsil, uterus and embryonic tissues. *MS4A7* ESTs were from lung, kidney, lymphocytes, mammary gland, placenta, spleen, testis, thymus, and uterine tissues. *MS4A8B* ESTs were from brain, lung, uterus and embryonic tissues. A single *MS4A12* EST was isolated from colon. This demonstrates differential *MS4A* gene transcription among lymphoid and non-lymphoid tissues.

Table 3

MS4A mRNA Expression by Human Lymphoblastoid Cell Lines

	MS4A family member[a]									
Cell lines:	1	2	3	4A	5	6A	7	8B	12	G3PDH
Pre-B:										
NALM-6	-	-	-	+++	-	-	-	-	-	+++
B cell:										
BJAB	+++	-	-	+++	-	-	+++	+	-	+++
DAUDI	+++	-	-	+	-	-	+++	+	-	+++
SB	+++	-	-	++	-	+++	+++	+	-	+++
T cell:										
HSB-2	-	-	-	+	-	-	-	-	-	+++

HUT-78	-	-	-	+	-	-	+	-	-	+++
JURKAT	-	-	-	+	-	-	-	-	-	+++
MOLT15	-	-	-	+	-	-	++	-	-	+++
Myelomonocyte:										
HL60	-	-	+++	++	-	+++	+++	-	-	+++
U937	-	-	+++	+++	+	+	+++	-	-	+++
Erythroleukemia:										
K562	-	+	+++	+++	-	+	-	-	-	+++

[a]Gene transcription was assessed by PCR amplification of cDNA generated from mRNA isolated from each cell type. Valu represent the level of PCR product generated relative to the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) control in thr separate PCR reactions: -, no specific PCR product detected; +, low levels of the appropriate band were detectable; ++ to +- appropriate bands of increasing intensity were readily visualized in all samples examined. Identical results were obtained using t different primer pairs for cDNA amplification.

Since most of the MS4A genes are expressed by hematopoietic cells, *MS4A4E, MS4A6E* and *MS4A10* transcription were assessed by RT-PCR amplification of cDNA from human hematopoietic cell lines and human tissues. Transcripts from eleven human hematopoietic cell lines were evaluated; one pre-B cell line (NALM-6), three B cell lines (BJAB, DAUDI, and SB), four T cell lines (HSB-2, HUT-78, JURKAT, and MOLT15), two myelomonocytic lines (HL60 and U937), and one erythroleukemia cell line (K562). In addition, transcripts from eight human tissues were evaluated; colon, ovary, peripheral blood leukocytes, prostate, small intestine, spleen, testes and thymus. However, *MS4A4E, MS4A6E* and *MS4A10* transcripts were not detected in any of these cell lines or tissues.

MS4A4E, *MS4A6E,* and *MS4A10* sequences were also used to search the translated GenBank databases using the BLAST program (Altschul et al., 1997). Eleven EST sequences representing *MS4A6E* transcripts were found that represented nine cDNAs isolated from pooled fetal organ libraries (GenBank Accession Nos. AA382998, AA909515, AA917066, AI222355, AI279944, AI684553, AI699419, AI743473, AI806247), one cDNA from a pooled germ cell tumor library (GenBank Accession No. AI968835), and one cDNA from a colon tumor (GenBank Accession No. AW951636). EST cDNAs encoding *MS4A4E* or *MS4A10* sequences were not identified. This suggests that *MS4A4E, MS4A6E,* and *MS4A10* transcripts are rare among normal tissues or they are primarily expressed during oncogenesis or embryogenesis.

MS4a gene expression by mouse tissues was assessed by Northern analysis and PCR amplification of cDNAs (Table 4). In most cases assessed, Northern analysis failed to detect specific MS4a transcripts in tissues that revealed transcript production by PCR amplification. These results suggest that MS4a transcripts are only produced by subpopulations of cells within each tissue such that transcript levels were often below the level of detection by Northern analysis. Nonetheless, *MS4a4B*, *MS4a4C*, and *MS4a6B* transcripts were found at high levels in thymus, spleen and peripheral lymph nodes, with less abundant levels in non-lymphoid tissues.

MS4a6C was only expressed by thymus, spleen, PLN and bone marrow. *MS4a4C*, *MS4a6D* and *MS4a7* were expressed in all tissues examined. *MS4a8B* transcripts were expressed by spleen, peripheral lymph nodes, colon, liver, heart, lung and bone marrow. *MS4a10* transcripts were found in thymus, kidney, colon, brain, and testis. In addition, CD20 (*MS4a1*), FcεRIβ (*MS4a2*), and *MS4a3* expression were primarily restricted to hematopoietic tissues. *MS4a3, MS4a4B, MS4a4C, MS4a6B, MS4a6C, MS4a6D, MS4a7, MS4a8B,* and *MS4a10* were also expressed by various hematopoietic and lymphoblastoid cell lines. Therefore, most MS4a family members were expressed by hematopoietic cells.

Table 4

MS4a Gene Expression by Mouse Tissues[a]

MS4a	Thymus	Spleen	PLN	BM	Liver	Kidney	Heart	Colon	Lung	Brain	Teste
1	+	+++	+++	+	-	-	-	-	+	-	-
2	+	+	+	+++	-	+	-	-	+	-	-
3	+	+	+	+++	-	-	-	-	+	+	-
4B	+++	+++	+++	++	+	+	+	+	+	-	-
4C	+++	+++	+++	+++	+	+	+	+	+	+	+
4D	+	+	++	-	+	+	++	++	++	-	+
6B	+++	+++	+++	++	+	-	+	+	+	-	++
6C	+	+	+	++	-	-	-	-	-	-	-
6D	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++
7	++	++	++	++	+	+	+	++	+	+	+
8B	-	+	+	+	+	-	+	++	+	-	-
10	+	-	-	-	-	+	-	+	-	+	++
G3PDH	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

[a]Gene transcription was assessed by PCR amplification of cDNA generated from mRNA isolated from tissue samples. Values represent the level of PCR product generated relative to the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) control as described for Table 3. Peripheral lymph node (PLN) and bone marrow (BM).

Expression of MS4A family members was also assessed in mouse hematopoietic cell lines (Table 5). Nine of the twelve MS4A genes were expressed in pre-B cell lines and five of the MS4A genes were expressed in B cell lines. Six of the MS4A genes were expressed by T cell lines. These data suggest that B cells can express most members of the MS4A gene family, although the patterns of expression of each gene is distinct.

Table 5

MS4a Expression by Mouse Lymphoid Tissues and Cell Lines[a]

	Tissues		Pre B cell lines			B cell lines		T cell lines	
MS4a	Spleen	Thymus	300.19	38B9	70Z	A20	AJ9	BW514	EL-14
1	+++	+	-	-	-	+++	+++	-	-
2	+	+	-	-	+	-	-	-	-
3	+	+	-	+	-	-	-	-	-
4B	+++	+++	-	-	-	-	-	-	++
4C	+++	+++	-	+	+	++	-	-	+
4D	+	+	-	-	-	-	-	-	-
6B	+++	+++	-	+	+++	+	-	+++	+++
6C	+	-	-	+	+	-	-	-	+
6D	+++	+++	-	++	+++	-	+	-	+++
7	++	++	-	-	++	-	-	-	-
8B	+	-	-	-	++	-	-	-	-
10	-	+	-	-	+	+	+	+	-
G3PDH	+++	+++	+++	+++	+++	+++	+++	+++	+++

[a]Gene transcription was assessed by PCR amplification of cDNA generated from mRNA isolated from each cell type. Values represent the level of PCR product generated relative to the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) control in three separate PCR reactions: -, no specific PCR product detected; +, low levels of the appropriate band were detectable; ++ to +++, appropriate bands of increasing intensity were readily visualized in all samples examined. Identical results were obtained using two different primer pairs for cDNA amplification.

III.B. Detection of MS4A Polymorphisms

In another embodiment, genetic assays based on nucleic acid molecules of the present invention can be used to screen for genetic variants by a number of PCR-based techniques, including single-strand conformation polymorphism (SSCP) analysis (Orita, M., et al. (1989) *Proc Natl Acad Sci USA* 86(8):2766-2770), SSCP/heteroduplex analysis, enzyme mismatch cleavage, and direct sequence analysis of amplified exons (Kestila et al. (1998) *Mol Cell* 1(4):575-582; Yuan et al. (1999) *Hum Mutat* 14(5):440-446). Automated methods can also be applied to large-scale characterization of single nucleotide polymorphisms (Brookes (1999) *Gene* 234(2):177-186; Wang et al. (1998) *Science* 280(5366):1077-82). The present invention further provides assays to detect a mutation of a variant MS4A locus by methods such as allele-specific hybridization (Stoneking et al. (1991) *Am J Hum Genet* 48(2):370-82), or restriction analysis of amplified genomic DNA containing the specific mutation.

IV. Recombinant Production of a MS4A Polypeptide

The present invention also provides a method for recombinant production of a MS4A polypeptide, as described in Example 3. Preferably, the recombinant polypeptide comprises some or all of the amino acid sequences of any one of the even-numbered SEQ ID NOs:2-38.

Recombinantly produced proteins are useful for a variety of purposes, including structural determination of a MS4A polypeptide, generation of an antibody that recognizes a MS4A polypeptide, and screening assays to identify a chemical compound or peptide that interacts with a MS4A polypeptide, described further herein below.

V. Production of MS4A Antibodies

In another aspect, the present invention provides a method of producing an antibody immunoreactive with a MS4A polypeptide, the method comprising recombinantly or synthetically producing a MS4A polypeptide, or portion thereof, to be used as an antigen. The MS4A

polypeptide is formulated so that it is can be used as an effective immunogen. An animal is immunized with the formulated MS4A polypeptide, generating an immune response in the animal. The immune response is characterized by the production of antibodies that can be collected from the blood serum of the animal. Optionally, cells producing a MS4A antibody can be fused with myeloma cells, whereby a monoclonal antibody can be selected. Exemplary methods for producing a monoclonal antibody that recognizes a MS4A protein are described in Example 4. Preferred embodiments of the method use a polypeptide set forth as any one of the even-numbered SEQ ID NOs:2-38.

The present invention also encompasses antibodies and cell lines that produce monoclonal antibodies as described herein.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the MS4A polypeptide sequences of the invention, *e.g.*, for cloning of MS4A nucleic acids, immunopurification of MS4A polypeptides, imaging MS4A polypeptides in a biological sample, measuring levels thereof in appropriate biological samples, and in diagnostic methods.

VI. Methods for Detecting a MS4A Polypeptide

In another aspect of the invention, a method is provided for detecting a level of MS4A polypeptide using an antibody that specifically recognizes a MS4A polypeptide, or portion thereof. In a preferred embodiment, biological samples from an experimental subject and a control subject are obtained, and MS4A polypeptide is detected in each sample by immunochemical reaction with the MS4A antibody. More preferably, the antibody recognizes amino acids of any one of the even-numbered SEQ ID NOs:2-38, and is prepared according to a method of the present invention for producing such an antibody.

In one embodiment, a MS4A antibody is used to screen a biological sample for the presence of a MS4A polypeptide. A biological sample to be screened can be a biological fluid such as extracellular or intracellular fluid,

or a cell or tissue extract or homogenate. A biological sample can also be an isolated cell (*e.g.*, in culture) or a collection of cells such as in a tissue sample or histology sample. A tissue sample can be suspended in a liquid medium or fixed onto a solid support such as a microscope slide. In accordance with a screening assay method, a biological sample is exposed to an antibody immunoreactive with a MS4A polypeptide whose presence is being assayed, and the formation of antibody-polypeptide complexes is detected. Techniques for detecting such antibody-antigen conjugates or complexes are well known in the art and include but are not limited to centrifugation, affinity chromatography and the like, and binding of a labeled secondary antibody to the antibody-candidate receptor complex.

In one embodiment, an antibody that specifically recognizes a MS4A polypeptide can be used to assess the tissue- or cell-distribution of MS4A protein, for example, to evaluate CD20 expression during B lymphocyte development (Figure 9). CD20 expression in $B220^{+}$ lymphocytes from lymphoid tissues of wild type mice was examined by two-color immunofluorescence. In bone marrow, three types of $B220^{+}$ cells were detected. The vast majority of $B220^{hi}$ lymphocytes expressed CD20. However, the majority of $B220^{lo}$ lymphocytes were CD20-negative. Thus, CD20 was predominantly expressed by mature B cells.

CD19 expression is restricted to normal and neoplastic B cells and follicular dendritic cells. CD19 is expressed early by B progenitor cells in the bone marrow, presumably at the late pro-B or early pre-B cell stages around the time of immunoglobulin heavy chain rearrangement (Anderson et al. (1984) *Blood* 63:1424). Expression persists during all stages of B cell maturation and is lost upon terminal differentiation to plasma cells.

Double staining of CD20 with IgM and CD19 antibodies showed that some of the $CD19^{lo}$ and IgM^{lo} cells were CD20 negative in the bone marrow. A few IgM- cells also expressed low levels of CD20 in the bone marrow. This data suggested that the CD20 expression was later than the CD19 expression but before or around the time of IgM expression during B cell

development in the bone marrow since these cells were gated on lymphocytes not dendritic cells.

The level of CD20 expression observed on mature B220^{hi} B cells in bone marrow was maintained by B cells from peripheral lymphoid tissues. The vast majority of B220^{+} B cells in the spleen, blood, peripheral lymph nodes, and peritoneal cavity expressed CD20. Therefore, like human CD20, mouse CD20 was also exclusively expressed on B cells from the immature B cell stage to mature B cells.

VII. Identification of MS4A Modulators

VII.A. Screening for Small Molecule Ligands that Interact with a MS4A Polypeptide

The present invention further discloses a method for identifying a compound that modulates MS4A function. According to the method, a MS4A polypeptide is exposed to a plurality of compounds, and binding of a compound to the isolated MS4A polypeptide is assayed. A compound is selected that demonstrates specific binding to the isolated MS4A polypeptide. Preferably, the MS4A polypeptide used in the binding assay of the method includes some or all amino acids of any one of the even-numbered SEQ ID NOs:2-38.

Several techniques can be used to detect interactions between a protein and a chemical ligand without employing an *in vivo* ligand. Representative methods include, but are not limited to, Fluorescence Correlation Spectroscopy, Surface-Enhanced Laser Desorption/Ionization Time-Of-flight Spectroscopy, and Biacore technology, as described in Example 5. These methods are amenable to automated, high-throughput screening.

Candidate regulators include but are not limited to proteins, peptides, and chemical compounds. Structural analysis of these selectants can provide information about ligand-target molecule interactions that enable the development of pharmaceuticals based on these lead structures.

Similarly, the knowledge of the structure a native MS4A polypeptide provides an approach for rational drug design. The structure of a MS4A polypeptide can be determined by X-ray crystallography or by computational algorithms that generate three-dimensional representations. <u>See</u> Huang et al. (2000) *Pac Symp Biocomput* 230-41; Saqi et al. (1999) *Bioinformatics* 15:521-522. Computer models can further predict binding of a protein structure to various substrate molecules, that can be synthesized and tested. Additional drug design techniques are described in U.S. Patent Nos. 5,834,228 and 5,872,011.

<u>VII.B. Methods for Identifying Modulators of MS4A Gene Expression</u>

The assembly and annotation of genomic sequences comprising MS4A genes in the region of human chromosome 11q12-13.1, disclosed herein for the first time, identify MS4A gene regulatory regions. Preferably, MS4A gene regulatory regions comprise sequences upstream of the initial coding region of each MS4A gene as disclosed in SEQ ID NOs:73-81. An expression cassette comprising a MS4A promoter region can be employed in assays for the identification of modulators of MS4A expression. Thus the present invention also provides a method for identifying a substance that regulates MS4A gene expression using a chimeric gene that includes an isolated MS4A gene promoter region operably linked to a reporter gene. According to this method, a gene expression system is established that includes the chimeric gene and components required for gene transcription and translation so that reporter gene expression is assayable. To select a substance that regulates MS4A gene expression, the method further provides the steps of using the gene expression system to determine a baseline level of reporter gene expression in the absence of a candidate regulator; providing one or more candidate regulators to the gene expression system; and assaying a level of reporter gene expression in the presence of a candidate regulator. A candidate regulator is selected whose presence results in an altered level of reporter gene expression when compared to the baseline level.

Several molecular cloning strategies can be used to identify

substances that specifically bind a MS4A gene cis-regulatory element. In one embodiment, a cDNA library in an expression vector, such as the lambda-gt11 vector, can be screened for cDNA clones that encode a MS4A gene regulatory element DNA-binding activity by probing the library with a labeled MS4A DNA fragment, or synthetic oligonucleotide (Singh et al. (1989) *Biotechniques* 7:252-261). Preferably, the nucleotide sequence selected as a probe has already been demonstrated as a protein binding site using a protein-DNA binding assay, as described in Example 9.

In another embodiment, transcriptional regulatory proteins are identified using the yeast one-hybrid system (Luo et al. (1996) *Biotechniques* 20(4):564-568; Vidal et al. (1996) *Proc Natl Acad Sci USA* 93(19):10315-10320; Li & Herskowitz (1993) *Science* 262:1870-1874). In this case, a cis-regulatory element of a MS4A gene is operably fused as an upstream activating sequence (UAS) to one, or typically more, yeast reporter genes such as the *lacZ* gene, the *URA3* gene, the *LEU2* gene, the *HIS3* gene, or the *LYS2* gene, and the reporter gene fusion construct(s) is inserted into an appropriate yeast host strain. It is expected that the reporter genes are not transcriptionally active in the engineered yeast host strain, for lack of a transcriptional activator protein to bind the UAS derived from the MS4A gene promoter region. The engineered yeast host strain is transformed with a library of cDNAs inserted in a yeast activation domain fusion protein expression vector, *e.g.* pGAD, where the coding regions of the cDNA inserts are fused to a functional yeast activation domain coding segment, such as those derived from the GAL4 or VP16 activators. Transformed yeast cells that acquire a cDNA encoding a protein that binds a cis-regulatory element of a MS4A gene can be identified based on the concerted activation the reporter genes, either by genetic selection for prototrophy (*e.g. LEU2, HIS3,* or *LYS2* reporters) or by screening with chromogenic substrates (e.g., a *lacZ* reporter) by methods known in the art.

The present invention also provides an *in vivo* assay for discovery of modulators of MS4A gene expression. In this case, a transgenic non-human animal is made such that a transgene comprising a MS4A gene promoter

and a reporter gene is expressed and a level of reporter gene expression is assayable. Such transgenic animals can be used for the identification of compounds that are effective in modulating MS4A gene expression.

In vitro or *in vivo* screening approaches can also survey more than one modulatable transcriptional regulatory sequence simultaneously.

VIII. Animal Models

The present invention further pertains to an animal model of disorders associated with a MS4A nucleic acid or polypeptide, including but not limited to atopic disorders, abnormal target cell development, function, and Ca^{++} responses. Such a model can be prepared by several methods. Using a transgenic approach, knock-out, knock-in, or knock-down mutation of the MS4A gene can suppress MS4A function. The present invention also teaches that an animal model of a MS4A-related disorder can be prepared by immunizing an animal with a MS4A polypeptide. The resulting immune response in the animal comprises a production of antibodies that specifically bind a MS4A polypeptide, thereby disrupting its biological activity. A method is also provided for generating an animal model of a MS4A-related disorder by administering to an animal a compound that disrupts MS4A expression or function. Such a compound is discovered by methods disclosed herein.

VIII.A. Generation of CD20-Deficient Mice

CD20-deficient mice were generated by targeted disruption of the *CD20* gene in embryonic stem (ES) cells using homologous recombination, as described in Example 6. A targeting vector was generated that replaces exons encoding part of the second extracellular loop, the 4th transmembrane domain, and the large carboxyl-terminal cytoplasmic domain of CD20 with a neomycin resistant gene (Figure 10A-D). Appropriate gene targeting generates an aberrant CD20 protein truncated at amino acid position 157 and fused with an 88 amino acid protein encoded by the Neo^r gene promoter sequence.

After DNA transfections, 6 of 115 Neo-resistant ES cell clones carried the targeted allele as determined by Southern blot analysis of EcoR V

digested genomic DNA using a 1.5 kb DNA probe (Figure 10D). Appropriate targeting was further verified in two clones by Southern analysis of ES cell DNA digested with BamH I (>12 kb fragment was reduced to a 6.5 kb band in targeted cells), Kpn I (7.2 kb became 5.5 kb), and Ssp I (5.6 kb became 7.0 kb) using the same probe. Cells of one ES cell clone were injected into blastocysts that were transferred into foster mothers. Highly chimeric male offspring (80-100% according to coat color) bred with C57BL/6 (B6) females transmitted the mutation to their progeny (Figure 10E). Mice homozygous for disruption of the *CD20* gene were obtained at the expected Mendelian frequency by crossing heterozygous offspring.

Appropriate targeting of the *CD20* gene was further verified by PCR analysis of genomic DNA from homozygous offspring (Figure 10F). Wild type CD20 mRNA was absent in $CD20^{-/-}$ mice as confirmed by PCR amplification of cDNA generated from splenocytes of $CD20^{-/-}$ mice (Figure 10G). CD20-deficient mice ($CD20^{-/-}$) thrived and reproduced as well as their wild type littermates and did not present any obvious anatomical or morphological abnormalities during the first year of life.

Absence of cell surface CD20 protein expression in $CD20^{-/-}$ mice was further verified by staining $B220^{+}$ splenocytes with murine anti-CD20 monoclonal antibodies. Hybridomas producing these antibodies were generated using splenocytes from $CD20^{-/-}$ mice that were immunized with CD20-GFP cDNA-transfected 300.19 cells. Ten hybridomas secreted antibodies reactive with 300.19 (Figure 10H) and CHO (Figure 10I) cells transfected with CD20-GFP cDNA, but not with untransfected CHO or 300.19 cells (Table 6). These antibodies also reacted with CD20 epitopes expressed on the cell surface of $B220^{+}$ splenocytes from wild type mice, but not with splenocytes from $CD20^{-/-}$ mice (Figure 10J). Therefore, targeted mutation of the *CD20* gene abrogated cell surface CD20 protein expression.

Table 6

Anti-CD20 Monoclonal Antibodies Generated in $CD20^{-/-}$ Mice[a]

			Whole Cell ELISA[a]		FACS Analysis[b]		
Ab Name	Clone Name	Isotype	CD20-CHO	CHO	CD20-300.19	300.19	Spleen
MB20-1	MCD20-5	IgG1, K	+	-	+	-	+
MB20-2	MCD20-61	IgG1, K	+	-	+	-	++
MB20-3	MCD20-86	IgG3, K	+	-	+	-	++
MB20-6	MCD20-223	IgG2a, K	+	-	+	-	+
MB20-7	MCD20-243	IgG2b, K	+	-	+	-	+
MB20-8	MCD20-270	IgG2b, K	+	-	+	-	+
MB20-10	MCD20-388	IgG2b, K	+	-	+	-	+
MB20-11	MCD20-392	IgG2a, K	+	-	+	-	+
MB20-13	MCD20-624	IgG3, K	+	-	+	-	++
MB20-14	MCD20-642	IgG1, K	+	-	+	-	++

[a]Values represent reactivity of the monoclonal antibody with adherent monolayers of CHO cells either transfected or untransfected with CD20-GFP cDNA as assessed by a cell-based ELISA. The monoclonal antibodies did not react with GFP cDNA-transfected CHO cells.

[b]Cell surface reactivity of the monoclonal antibody with single cell suspensions of 300.19 cells either transfected or untransfected with CD20-GFP cDNA or spleen cells from wild type mice. Values represent relative indirect immunofluorescence staining intensity as assessed by flow cytometry and shown in figure 10H-J.

VIII.B. B Cell Development and Function in $CD20^{-/-}$ Mice

$CD20^{-/-}$ mice did not show an obvious propensity for infections during their first year of life. They had normal frequencies of IgM^- $B220^{lo}$ pro/pre-B cells, IgM^+ $B220^{lo}$ immature B cells and IgM^+ $B220^{hi}$ mature B cells in the bone marrow (Figure 11, Table 7). Overall, the number of circulating and spleen IgM^+ $B220^+$ B cells found in $CD20^{-/-}$ mice was increased compared with wild type littermates (Table 7). However, an immunohistochemical analysis of spleen tissue sections revealed a normal architecture and organization of the spleen. In the bone marrow, overall IgM expression was decreased on immature B cells, yet increased on mature B cells when compared with IgM levels expressed by comparable cells in wild type littermates. However, overall IgM expression by mature $B220^{hi}$ B cells in the blood, spleen and lymph nodes was slightly lower in $CD20^{-/-}$ mice (Figure 11B-D). There were no obvious differences in the size (light scatter properties) of $CD20^{-/-}$ B cells isolated from bone marrow, blood, lymph nodes or spleen when compared with B cells from wild type littermates. These data therefore suggest that CD20 plays a functional role in the development and tissue localization of B cells.

Table 7

Frequencies and Numbers of B Lymphocytes in $CD20^{-/-}$ Mice

Tissue	Phenotype	Wild Type	$CD20^{-/-}$	Wild Type	CD20
		% of B Lymphocytes		B cell numbers ($\times 10^{-6}$	
Bone Marrow	$B220^{lo}IgM^{-}$	36 ± 2	34 ± 3		
	$B220^{lo}IgM^{+}$	19 ± 2	13 ± 2*		
	$B220^{hi}IgM^{+}$	14 ± 2	16 ± 4		
Blood[d]	$B220^{+}IgM^{+}$	61 ± 2	60 ± 3	3.6 ± 0.5	3.9 ±
Spleen	$B220^{+}IgM^{+}$	51 ± 6	53 ± 5	58 ± 12	76 ±
Lymph Nodes[e]	$B220^{+}IgM^{+}$	26 ± 6	19 ± 2	1.2 ± 0.3	0.9 ±
Peritoneum	$B220^{+}IgM^{+}$	70 ± 4	69 ± 5	2.4 ± 0.3	3.1 ±
	$B220^{lo}CD5^{+}$	44 ± 4	15 ± 5**	1.5 ± 0.2	0.7 ± (
	$B220^{hi}CD5^{-}$	28 ± 2	59 ± 3**	1.0 ± 0.1	2.7 ± (

[a]Values represent mean (± SEM) results obtained from seven 2-month-old of wild type controls and 10 $CD20^{-/-}$ mice. Numbers represent the percentage of lymphocytes (based on side and forward light scatter properties) expressing the indicated cell surface markers.

[b]B cell numbers were calculated based on the total number of cells harvested from the indicated tissues.

[d]The values indicate the number of cells/ml.

[e]Values represent results from peripheral lymph nodes pairs.

*The percentage or number was significantly different than in wild-type, $p < 0.05$; ** $p < 0.01$.

Within the peritoneal cavity, the number of IgM^+ $B220^+$ B cells in $CD20^{-/-}$ mice was similar to that of wild-type littermates (Table 7, Figure 11E). However, there was a 4-fold decrease in the number of $CD5^+$ $B220^{lo}$ B1a cells, with a compensatory increase in the number of $CD5^-$ $B220^{hi}$ B2 cells. Therefore, CD20-deficiency predominantly affected the development or clonal expansion of the B1 subpopulation of B cells within the peritoneal cavity. Exemplary methods for quantitating B cell populations are described in Example 7.

VIII.C. Reduced $[Ca^{++}]i$ Responses in $CD20^{-/-}$ B Cells

The loss of CD20 significantly altered early B cell signaling responses, measured as described in Example 8. Splenic $B220^+$ B cells from $CD20^{-/-}$ mice generated substantially reduced $[Ca^{++}]i$ responses following surface IgM ligation when compared with wild type B cells. Decreased $[Ca^{++}]i$ responses in $CD20^{-/-}$ B cells were observed in response to both optimal (40 µg/ml, Figure 12A) and suboptimal concentrations (5 µg/ml) of anti-IgM antibodies. Although the kinetics of $[Ca^{++}]i$ responses in $CD20^{-/-}$ B cells was not altered, the magnitude of both the immediate $[Ca^{++}]i$ increase and the sustained increase observed at later time points were inhibited by loss of CD20 expression. More dramatic decreases in $[Ca^{++}]i$ responses (>50%) by $CD20^{-/-}$ B cells were observed in response to CD19 ligation with optimal concentrations (40 µg/ml) of antibody (Figure 12A). Reduced $[Ca^{++}]i$ responses following CD19 ligation on $CD20^{-/-}$ B cells were likely to result from differences in signaling capacity since Thapsigargin-induced (Figure 12A) and Ionomycin-induced $[Ca^{++}]i$ responses were higher in $CD20^{-/-}$ B cells than in wild type B cells. In addition, CD19 expression levels were not significantly different between $CD20^{-/-}$ and wild type B cells (Figure 12A).

Chelation of extracellular calcium with EGTA reduced the kinetics and magnitude of the immediate $[Ca^{++}]i$ increase observed following IgM

crosslinking (Figure 12A). However, the $[Ca^{++}]_i$ increase observed at later time points was not substantially inhibited by EGTA treatment. Similar results were observed in $CD20^{-/-}$ B cells. By contrast, chelation of extracellular calcium with EGTA almost eliminated the $[Ca^{++}]_i$ response observed following CD19 crosslinking (Figure 12A). This suggests that transmembrane Ca^{++} flux contributes substantially to the $[Ca^{++}]_i$ responses observed following CD19 crosslinking. That CD20-deficiency had a substantial effect on CD19-induced $[Ca^{++}]_i$ responses suggests that CD20 can contribute significantly to transmembrane Ca^{++} flux.

The consequences of CD20 loss on transmembrane signal transduction was further evaluated by assessing total cellular protein tyrosine phosphorylation in purified B cells following IgM ligation. Although some variation was observed between B cells from individual mice in individual experiments, overall levels of tyrosine phosphorylation in resting splenic B cells were higher in $CD20^{-/-}$ B cells than in wild type mice (Figure 12C). In addition, protein phosphorylation in B cells from $CD20^{-/-}$ mice increased more significantly after B cell antigen receptor (BCR) ligation than in wild type B cells. Thus, while CD20 expression can influence BCR-induced tyrosine phosphorylation, decreased $[Ca^{++}]_i$ responses in $CD20^{-/-}$ B cells are unlikely to result from significant abnormalities in transmembrane signaling through the BCR.

IX. Therapeutic Applications

Another aspect of the present invention is a therapeutic method comprising administering to a subject a substance that modulates MS4A biological activity. Therapeutic substances include but are not limited to chemical compounds, antibodies, and gene therapy vectors. Substances that are discovered by the methods disclosed herein are useful for therapeutic applications related to disorders of MS4A function.

In one embodiment, the present invention provides a method for disrupting MS4A function by immunizing a subject with an effective dose of the disclosed MS4A polypeptide. The immune system of the subject produces an antibody that specifically recognizes the MS4A polypeptide, and binding of the antibody to the MS4A polypeptide abolishes MS4A function.

In another embodiment, the present invention provides MS4A nucleic acid sequences and gene therapy methods for modulating MS4A activity in a target cell. The gene therapy vector can encode a MS4A or sequences encoding a nucleic acid molecule, peptide, or protein that interacts with a MS4A protein.

Vehicles for delivery of a gene therapy vector include but are not limited to a liposome, a cell, and a virus. Preferably, a cell is transformed or transfected with the DNA molecule or is derived from such a transformed or transfected cell. Alternatively, the vehicle is a virus, including a retroviral vector, adenoviral vector or vaccinia virus whose genome has been manipulated in alternative ways so as to render the virus non-pathogenic. Methods for creating such a viral mutation are detailed in U.S. Patent No. 4,769,331. Exemplary gene therapy methods are also described in U.S. Patent Nos. 5,279,833; 5,286,634; 5,399,346; 5,646,008; 5,651,964; 5,641,484; and 5,643,567.

The therapeutic methods of the present invention can be applied in the treatment of a variety of conditions, including in the treatment of non-Hodgkin's lymphoma and in the treatment of atopic disorders or other allergenic diseases. Application of the present inventive therapeutic methods are evidenced by the current U.S. Food and Drug Administration approved use of antibodies against CD20 in the treatment of non-Hodgkin's lymphoma. Additionally, the therapeutic methods of the present invention are illustrated in view of the recognition in the art that genetic variations at chromosome 11Q12-13 can also play a role in the pathogenesis of atopic disorders and other allergenic diseases. Indeed, it has been recognized that

FcεRIβ contributes to such diseases, and thus the MS4A genes identified in accordance with the present invention are envisioned also to contribute to allergenic disease. Therefore the present therapeutic methods, which pertain to the modulation of the biological activity of an MS4A polypeptide of the present invention have application with respect to the treatment of such disorders.

X. Summary

The invention comprises 19 new genes that are members of a class of genes encoding MS4A proteins. Three members have been described, CD20, FcεRIβ, and HTm4. A gene family has been defined based on a shared chromosomal location, conservation of protein size and structure, gene structure conservation, and similar expression in hematopoietic cells. MS4A proteins function as oligomeric cell surface complexes, and complex assembly using diverse MS4A members is implicated as a mechanism for regulating complex function.

Two members of this class, CD20 and FcεRIβ, have been described functionally, and in each case an important function has been delineated. CD20 is required for cell cycle progression and signal transduction in B lymphocytes. CD20 also regulates Ca^{++} conductance, possibly as a cation channel subunit. Of clinical relevance, antibodies that recognize CD20 are effective in treating non-Hodgkin's lymphoma. FcεRIβ mediates interactions with IgE-bound antigens that lead to degranulation of mast cells, and variation of the FcεRIβ locus is implicated in allergenic disease.

The utility of the MS4A genes is based in part on overlapping or shared functions with known MS4A members. In one case, new MS4A genes have important potential as part of a CD20 complex. The structural description of CD20 complexes suggests that one or more CD20-related proteins constitute the functional complex. Thus, new MS4A proteins can define antigens useful for lymphoma treatment. In another case, MS4A

genes are implicated in IgE responses. Atopic disorders (allergy, asthma, eczema, allergic rhinitis) are dysfunctional IgE responses and are associated with a locus on human chromosome 11q containing most members of the MS4A gene family. FcεRIβ is one relevant factor, and recent work supports that FcεRIβ as well as other genetic elements in the region contribute to the disease. Thus, as disclosed herein, the present MS4A sequences also have utility in the characterization, diagnosis, and potential treatment of atopy linked to the chromosomal location wherein MS4A genes are located.

Examples

The following Examples have been included to illustrate modes of the invention. Certain aspects of the following Examples are described in terms of techniques and procedures found or contemplated by the present co-inventors to work well in the practice of the invention. These Examples illustrate standard laboratory practices of the co-inventors. In light of the present disclosure and the general level of skill in the art, those of skill will appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the invention.

Example 1

Database Searches and cDNA Isolation

Three hundred and thirty seven nucleotide sequences obtained from the translated GenBank database of expressed sequence tags (ESTs) were assembled into sixty-two subgroups of contiguous linear segments based on their overlapping sequences and potential for encoding proteins homologous with CD20. Based on these subgroups, EST cDNAs (Figure 1) were obtained from the ATCC and sequenced. Based on the complete sequences of twenty-one near full-length EST cDNAs, eleven novel genes were defined in human and mouse that unified multiple EST subgroups. Near full-length EST clones representing these genes are shown in Figure 1.

These eleven genes and five additional genes were also identified by PCR amplification of transcripts using subgroup-specific primers or primers based on EST sequences. The specific details of how cDNAs representing the five genes that were not identified by EST cDNA clones are indicated below. In all cases, ESTs and cDNAs encoding the predicted coding regions of each putative unique gene were sequenced in both directions and at least two independent ESTs and/or cDNAs representing near full-length gene products were sequenced. Thereby, there was independent confirmation of accuracy for all of the sequences reported.

Based on EST subgroup sequences, cDNAs encoding mouse *MS4a4B* and *MS4a4C* were isolated by PCR amplification of C57BL/6 mouse spleen cDNA using both Taq and Pfu DNA polymerase. Primers for *MS4a4B* (SEQ ID NOs:63-64) amplified an 879 bp fragment. Primers for *MS4a4C* (SEQ ID NOs:65-66) amplified a 794 bp fragment. EST sequences for *MS4a4D* only encoded the 3' end of the predicted protein. Since *MS4a4D* sequences were closely related to *MS4a4B* and *MS4a4C* sequences, a sense 5' primer (SEQ ID NO:67) based on consensus *MS4a4B* and *MS4a4C* sequences and a *MS4a4D*-specific antisense primer (SEQ ID NO:68) were used to amplify a 773 bp fragment from cDNA of C57BL/6 mouse lung.

MS4a6C was initially identified based on one unique EST sequence (AA028258) encoding a mouse protein homologous with the C-terminal end of MS4a6B. *MS4a6C* cDNAs were isolated by PCR amplification of C57BL/6 mouse bone marrow cDNA using Taq polymerase. A primer based on identical sequences at the 5' end of the *MS4a6B* and *MS4a6D* cDNAs (SEQ ID NO:69) was used in combination with an antisense primer specific for the unique EST sequence (SEQ ID NO:70) to amplify a 787 bp fragment. Sequences from multiple independent PCR-amplified cDNAs were identical. Subsequently, the PCR-generated 5' end of the near full-length *MS4a6C* cDNA was found to be identical to an orphan EST subgroup sequence that had not been linked with defined 3' sequences. Thereby, the EST subgroup sequences verified that the PCR-amplified 5' end of the *MS4a6C* cDNAs

was appropriate. In addition, the overall *MS4a6C* sequence was similar to the sequence of *MS4a6B* cDNAs without interruption. Thus, the *MS4a6C* cDNA united sequences identical to those found in two non-overlapping CD20-homologous EST subgroups. cDNAs encoding a 473 bp fragment of mouse *MS4a3* were amplified from cDNA of C57BL/6 bone marrow as described above. Primers (SEQ ID NOs:71-72) were obtained based on a single thymic cDNA EST sequence (GenBank AA940479) where the corresponding cDNA was not available.

Human MS4A and mouse MS4a cDNA sequences (MS4A1 to MS4A12) (disclosed herein) were used to search the htgs GenBank human genomic database of unfinished human genomic sequences (http://www.ncbi.nlm.nih.gov/blast/) using the BLAST program. Seventeen phase 1 or phase 2 human genomic DNA sequences encoding potential MS4A genes were assembled into groups of contiguous linear segments based on their overlapping sequences. Three EST clones corresponding to partial *MS4A6E* transcripts were obtained from the ATCC and sequenced completely on both DNA stands.

All PCR-amplified cDNAs were subcloned and sequenced entirely in both directions. Complete sequencing of at least two distinct PCR-generated cDNAs from both Taq and Pfu enzyme was performed in most cases. Differences between cDNA sequences were only noted when multiple cDNA clones generated by both Taq and Pfu polymerases revealed identical differences. In some cases, cDNAs or EST sequences contained potential intron|exon splice sites that delimited structural domains and aligned with the known intron|exon splice sites of CD20 (Tedder et al. (1989b) *J Immunol* 142:2560-2568). In these cases, potential introns were flanked by consensus splice donor and/or splice acceptor sequences (Aebi & Weissmann (1987) *Trends Genet* 3:102-107) or were likely to represent splice variants where exons were deleted.

Example 2

RNA Isolation and Reverse Transcription-PCR

Reverse transcription-PCR amplification (RT-PCR) was as described previously (Zhou & Tedder, 1995) with minor modifications. Total RNA was extracted from 1-2 x 10^7 hematopoietic cell lines using a RNeasy Mini Kit (Qiagen, Inc., Chatsworth, California) according to the manufacturer's instructions. Human hematopoietic cell lines included one pre-B cell line (NALM-6), three B cell lines (BJAB, DAUDI, and SB), four T cell lines (HSB-2, HUT-78, JURKAT, and MOLT15), two myelomonocytic lines (HL60 and U937), and one erythroleukemia cell line (K562). RNA concentrations were determined by UV absorbance. Ten μg of total RNA was reverse transcribed. In some cases, cDNA from any of 8 different human tissues (colon, ovary, blood mononuclear cells, prostate, small intestine, spleen, testes, and thymus; from CLONETECH Laboratories, Inc., Palo Alto, California) was analyzed. RT-PCR amplification was performed using gene-specific primers identical with protein coding regions of the predicted MS4A genes during 35 cycles (94°C for 1 min, 55°C for 1.5 min, 72°C for 1.5 min, followed by extension at 72°C for 5 min). Following amplification, the PCR products were separated on 1% agarose-ethidium bromide gels and photographed. G3PDH, a housekeeping gene, was also amplified to control for sample to sample variation. RNA amplified without reverse transcription was used as a negative control, and was negative in all cases.

Example 3

Recombinant Production of MS4A Protein

For recombinant production of a protein of the invention in a host organism, a nucleotide sequence encoding the protein is inserted into an expression cassette designed for the chosen host and introduced into the host where it is recombinantly produced. The choice of the specific regulatory sequences such as promoter, signal sequence, 5' and 3' untranslated sequence, and enhancer appropriate for the chosen host is within the level of ordinary skill in the art. The resultant molecule, containing

the individual elements linking in the proper reading frame, is inserted into a vector capable of being transformed into the host cell. Suitable expression vectors and methods for recombinant production of proteins are well known for host organisms such as *E. coli*, yeast, and insect cells (see, e.g., Lucknow & Summers (1988) *Bio/Technol* 6:47). Additional suitable expression vectors are baculovirus expression vectors, e.g., those derived from the genome of *Autographica californica* nuclear polyhedrosis virus (AcMNPV).

Recombinantly produced proteins are isolated and purified using a variety of standard techniques. The actual techniques used varies depending upon the host organism used, whether the protein is designed for secretion, and other such factors. Such techniques are well known to the skilled artisan. See Ausubel et al. (1994).

Example 4

Mouse Anti-Mouse CD20 Monoclonal Antibody Production

Hybridomas producing CD20-specific mouse monoclonal antibodies were generated by the fusion of NS-1 myeloma cells with spleen cells from a $CD20^{-/-}$ mouse immunized with a cell line expressing a mouse CD20-GFP fusion protein. The CD20-GFP fusion protein was generated by subcloning a fragment of the pmB1-1 cDNA (from 159 to 1050 bp of SEQ ID NO:39) into the PEGFP-N1 vector (Clonetech Laboratories Inc., Palo Alto, California) to generate an open reading frame encoding the entire CD20 protein with GFP fused to the carboxyl-terminal end. The resulting plasmid was linearized with ApaL I and used to transfect 300.19 cells, a mouse pre-B cell line, and Chinese Hamster Ovary (CHO) cells. Transfection was by Lipofectamine following the manufacturer's instructions (Clonetech Laboratories, Inc.). Transfected cells were selected using GENETICIN™ (1 mg/ml, GIBCOBRL) in RPMI 1640 media (Sigma) for 300.19 cells or H-12 nutrient mixture (GIBCOBRL) for CHO cells. Both media were supplemented with 10% FCS, L-glutamine, streptomycin and penicillin. Transfected cells expressing high levels of CD20-GFP were isolated by fluorescence-based cell sorting.

Example 5

In vitro Binding Assays

Recombinant protein can be obtained, for example, according to the approach described in Example 4 herein above. The protein is immobilized on chips appropriate for ligand binding assays. The protein immobilized on the chip is exposed to sample compound in solution according to methods well known in the art. While the sample compound is in contact with the immobilized protein, measurements capable of detecting protein-ligand interactions are conducted. Measurement techniques include, but are not limited to, SEDLI, Biacore, and FCS, as described above. Compounds found to bind the protein are readily discovered in this approach and are subjected to further characterization.

Example 6

Generation of CD20-Deficient Mice

DNA encoding the *CD20* gene was isolated from a phage library prepared from 129/Sv strain mouse DNA (Figure 10A), mapped with restriction endonucleases, and sequenced to identify intron|exon boundaries (Figure 10B). The targeting vector was constructed using a pBluescript SK (Stratagene, La Jolla, California)-based targeting vector (p594, provided by Dr. David Milstone, Brigham and Women's Hospital, Boston, Massachusetts). A DNA fragment starting at the Pst I site in *CD20* exon 5 through the EcoR V site in exon 6 (~1.8 kb) was isolated and blunt end ligated into the targeting vector downstream of the pMC1-HSV thymidine kinase gene and upstream of the neomycin resistance marker obtained from pGK-neo poly A (Stratagene) that contained the PGK promoter and poly A signal sequence. An ~10 kb DNA fragment beginning at the Kpn I site downstream of exon 8 was also isolated and inserted into the targeting vector downstream of the neomycin resistant gene. The plasmid was linearized using a unique Sal I restriction site proximal to the 3' end of the *CD20* gene insert and used to transfect ES cells.

ES cells were transfected with linearized plasmid DNA and selected for G418 resistance as described (Keller and Smithies (1989) *Proc Natl Acad Sci USA* 886:8932). Genomic DNA from individual selected clones was digested with EcoR V and used for Southern blot analysis along with a radiolabeled ~1.5 kb DNA probe that was external to the targeting vector (Figure 10D). A 4.6 kb genomic DNA fragment hybridized with the probe in wild type ES cells or a 6.3 kb fragment in appropriately targeted ES cells (Fig 1E). Genomic DNA generated by BamH I, Ssc I or Kpn I digestion was also analyzed for appropriate targeting. The Southern blot pattern obtained in all cases was consistent with the appropriate predicted mutation indicating that detrimental recombinations did not occur in the vicinity of the desired homologous recombination. Cells from appropriately targeted ES cell clones were injected into 3.5 day old C57BL/6 blastocysts that were transferred into foster mothers. Offspring carrying the mutant CD20 allele were identified by Southern blot analysis of DNA obtained from tail biopsies.

High chimeric males (80-100% according to color) were bred with C57BL/6 (B6) females to generate heterozygous offspring with germline gene transmission, which were crossed to generate the homozygous $CD20^{-/-}$ and wild type littermates used for this study. In some cases, B6/129F1J (Jackson Laboratory) were used as controls. Results obtained using wild type littermates of $CD20^{+/-}$ mice were similar and were therefore pooled. All mice were between 2-3 months of age when used for this study. Mice were housed in a specific pathogen-free barrier-facility. All studies and procedures were approved by the Animal Care and Use Committee of Duke University.

Example 7

Flow Cytometric Analysis of Lymphocyte Subsets

Single cell suspensions of lymphocytes from the spleen, bone marrow, peripheral lymph nodes, and peritoneal cavity were isolated from $CD20^{-/-}$ and wild type mice and counted using a hemocytometer prior to two-color immunofluorescence analysis. Retroorbital venous plexus puncture

was utilized to obtain circulating leukocytes. Leukocytes (0.5 x 10^6) were stained at 4°C using predetermined optimal concentrations of the test monoclonal antibody for 20 min as described (Zhou et al. (1994) *Mol Cell Biol* 14:3884-3894). Blood erythrocytes were lysed after staining using the Coulter Whole Blood Immuno-Lyse kit as detailed by the manufacturer (Coulter, Inc., Miami, Florida). Cells were washed and analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, California).

Antibodies used in this study included the following: biotin, FITC-conjugated anti-B220 Mab (CD45RA, RA-3, 6B2, provided by Dr. Robert Coffman, DNAXCORP, Palo, Alto, California); PE-conjugated anti-mouse Thy1.2 (Caltag Laboratories, Burlingame, California); B220-PE (Caltag Laboratories, Burlingame, California); biotin-conjugated anti-I-A (BD PharMingen, Franklin Lakes, New Jersey); PE or APC-conjugated anti-CD5 (BD PharMingen); PE-conjugated goat anti-mouse IgG3-specific antibody (Southern Biotechnology Associates Inc., Birmingham, Alabama); and biotin-conjugated anti-mouse IgD (Southern Biotechnology Associates Inc., Birmingham, Alabama). FITC or biotin-conjugated goat anti-mouse IgM isotype-specific antibodies (Southern Biotechnology Associates Inc., Birmingham, Alabama) were also used.

Phycoerythrin-conjugated Streptavidin (Southern Biotechnology Associates Inc., Birmingham, Alabama) was used to reveal biotin-coupled monoclonal antibody staining. The percent positively stained lymphocytes was determined using a FACScan flow cytometer (Becton Dickinson, San Jose, California). Positive and negative populations of cells were determined by using unreactive monoclonal antibody (Caltag Laboratories, Burlingame, California) as controls for background staining. Background levels of staining were delineated using gates positioned to include 98% of the control cells. Ten thousand cells with the forward and side light scatter properties of lymphocytes were analyzed for each sample.

Example 8

Intracellular Calcium Measurements

Changes in lymphocyte $[Ca^{2+}]_i$ levels were monitored by flow cytometry analysis as described (Fujimoto et al. (1999) *Immunity* 11:191). Single cell suspension of splenocytes were resuspended (1×10^7/ml) in RPMI 1640 medium containing 5% FBS, 10 mM HEPES and loaded with 1μM of indo-1-AM for 30 min at 37°C. Splenocytes were then washed and incubated with a predetermined optimal concentration of FITC-conjugated anti-B220 monoclonal antibody for 15 min at room temperature. The splenocytes were washed again and resuspended at 2×10^6/ml in medium. The fluorescence ratio (405/525 nm) of $B220^+$ splenic B cells was monitored by flow cytometry at baseline for 1 min and for 6 min after stimulation with optimal and suboptimal concentrations of goat $F(ab')_2$ anti-IgM antibody (5-40 μg/ml), optimal concentrations of anti-mouse CD19 monoclonal antibody (40 μg/ml), Thapsigargin (1 μg/ml; Sigma), or Ionomycin (2.67 μg/ml; Calbiochem Biosciences, Inc., La Jolla, California). In some cases, EGTA (5 mM final; pH 7.0) was added to the cells, immediately followed by stimulation with the inducing agents described above. Results were plotted as the fluorescence ratio at 20 sec intervals with background fluorescence subtracted. An increase in the fluorescence ratio indicates an increase in $[Ca^{2+}]_i$.

Example 9

Characterization of a MS4A Promoter Region

A preferred *in vitro* technique for evaluating MS4A promoter function is a transient transfection assay. According to this method, one or more chimeric reporter genes comprising a MS4A promoter region is introduced into a relevant host cell (*e.g.*, a hematopoietic cell), and the resulting level of reporter gene expression is quantitated. Representative methods for making an expression system comprising a promoter region operably linked to a heterologous reporter sequence are disclosed in U.S. Patent No. 6,087,111.

To analyze the function of a MS4A promoter region *in vivo*, transgenic

mice bearing a chimeric gene comprising a MS4A promoter region are generated, and a level of reporter gene expression in each mouse is determined.

Within a candidate promoter region or response element, the presence of regulatory proteins bound to a nucleic acid sequence can be detected using a variety of methods well known to those skilled in the art (Ausubel et al., 1992). Briefly, *in vivo* footprinting assays demonstrate protection of DNA sequences from chemical and enzymatic modification within living or permeabilized cells. Similarly, *in vitro* footprinting assays show protection of DNA sequences from chemical or enzymatic modification using protein extracts. Nitrocellulose filter-binding assays and gel electrophoresis mobility shift assays (EMSAs) track the presence of radiolabeled regulatory DNA elements based on provision of candidate transcription factors. Computer analysis programs, for example TFSEARCH version 1.3 (Yutaka Akiyama: "TFSEARCH: Searching Transcription Factor Binding Sites", http://www.rwcp.or.jp/papia/), can also be used to locate consensus sequences of known cis-regulatory elements within a genomic region.

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The publications and other materials listed below and/or set forth in the text above to illuminate the background of the invention, and in particular cases, to provide additional details respecting the practice, are incorporated herein by reference. Materials used herein include but are not limited to the following listed references.

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It will be understood that various details of the invention can be changed without departing from the scope of the invention. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation--the invention being defined by the claims.

CLAIMS

What is claimed is:

1. An isolated MS4A polypeptide, or functional portion thereof, comprising:

(a) a polypeptide encoded by the nucleotide sequence of any one of the odd-numbered SEQ ID NOs:1-37;

(b) a polypeptide encoded by a nucleic acid molecule that is substantially identical to any one of the odd-numbered SEQ ID NOs:1-37;

(c) a polypeptide having the amino acid sequence of any one of the even-numbered SEQ ID NOs:2-38;

(d) a polypeptide that is a biological equivalent of the polypeptide of any one the even-numbered SEQ ID NOs:2-38; or

(e) a polypeptide which is immunologically cross-reactive with an antibody that shows specific binding with a polypeptide of any one of the even-numbered SEQ ID NOs:2-38.

2. An isolated nucleic acid molecule encoding a MS4A polypeptide, comprising:

(a) the nucleotide sequence of any one of the odd-numbered SEQ ID NOs:1-37; or

(b) a nucleic acid molecule substantially identical to any one of the odd-numbered SEQ ID NOs:1-37.

3. The isolated nucleic acid molecule of claim 2, comprising a 20 nucleotide sequence that is identical to a contiguous 20 nucleotide sequence of any one of the odd-numbered SEQ ID NOs:1-37.

4. A chimeric gene, comprising the nucleic acid molecule of claim 2 operably linked to a heterologous promoter.

5. A vector comprising the chimeric gene of claim 4.

6. A host cell comprising the chimeric gene of claim 4.

7. The host cell of claim 6, wherein the cell is selected from the group consisting of a bacterial cell, a hamster cell, a mouse cell, and a human cell.

8. A method of detecting a nucleic acid molecule that encodes a MS4A polypeptide, the method comprising:

(a) procuring a biological sample comprising nucleic acid material;

(b) hybridizing the nucleic acid molecule of claim 2 under stringent hybridization conditions to the biological sample of (a), thereby forming a duplex structure between the nucleic acid of claim 2 and a nucleic acid within the biological sample; and

(c) detecting the duplex structure of (b), whereby a MS4A nucleic acid molecule is detected.

9. An antibody that specifically recognizes a MS4A polypeptide of claim 1.

10. A method for producing an antibody that specifically recognizes a MS4A polypeptide, the method comprising:

(a) recombinantly or synthetically producing a MS4A polypeptide, or portion thereof;

(b) formulating the polypeptide of (a) whereby it is an effective immunogen;

(c) administering to an animal the formulation of (b) to generate an immune response in the animal comprising production of antibodies, wherein antibodies are present in the blood serum of the animal; and

(d) collecting the blood serum from the animal of (c), the blood serum comprising antibodies that specifically recognize a MS4A polypeptide.

11. A method for detecting a level of MS4A polypeptide, the method comprising

(a) obtaining a biological sample comprising peptidic material; and

(b) detecting a MS4A polypeptide in the biological sample of (a) by immunochemical reaction with the antibody of claim 9, whereby an amount of MS4A polypeptide in a sample is determined.

12. A method for identifying a substance that modulates MS4A function, the method comprising:

(a) isolating a MS4A polypeptide of claim 1;

(b) exposing the isolated MS4A polypeptide to a plurality of substances;

(c) assaying binding of a substance to the isolated MS4A polypeptide; and

(d) selecting a substance that demonstrates specific binding to the isolated MS4A polypeptide.

13. A method for modulating MS4A function in a subject, the method comprising:

(a) preparing a pharmaceutical composition, comprising a substance identified according to the method of claim 10 or 12, and a carrier; and

(b) administering an effective dose of the pharmaceutical composition to a subject, whereby MS4A activity is altered in the subject.

14. The method of claim 13, wherein the substance is an antibody, a protein, a peptide, or a chemical compound.

15. The method of claim 13, wherein MS4A activity is regulation of the abundance of target cell subpopulations.

16. The method of claim 13, wherein MS4A activity is regulation of $[Ca^{2+}]_i$ levels.

17. A method for identifying a candidate compound as a modulator of MS4A gene expression, the method comprising:

(a) exposing a cell sample with a candidate compound to be tested, the cell sample containing at least one cell containing a DNA construct comprising a modulatable transcriptional regulatory sequence of a MS4A-encoding nucleic acid and a reporter gene which is capable of producing a detectable signal;

(b) evaluating an amount of signal produced in relation to a control sample; and

(c) identifying a candidate compound as a modulator of MS4A gene expression based on the amount of signal produced in relation to a control sample.

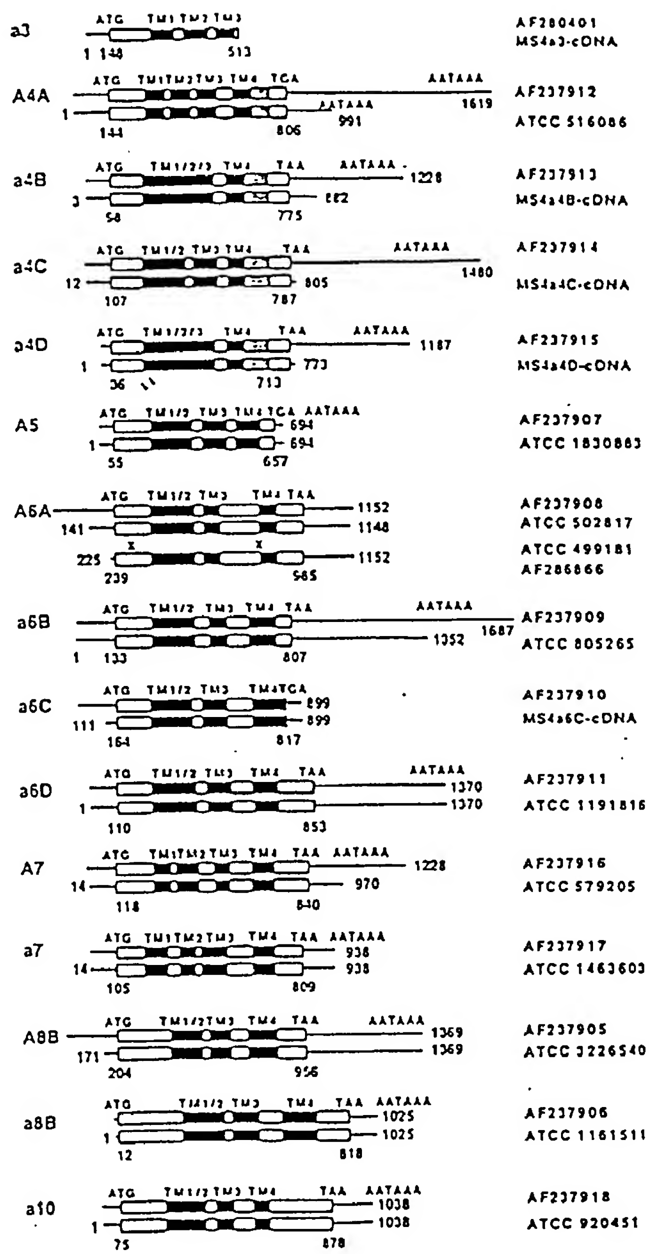
18. The method of claim 17, wherein the modulatable transcriptional regulatory sequence of a MS4A-encoding nucleic acid comprises a sequence that is immediately upstream of the initial coding region of a MS4A gene as set forth in any one of SEQ ID NOs:73-81.

19. A method for modulating MS4A function in a subject, the method comprising:

(a) preparing a gene therapy vector having a nucleotide sequence encoding a MS4A polypeptide or a nucleotide sequence encoding a nucleic acid molecule, peptide, or protein that interacts with a MS4A nucleic acid or polypeptide; and

(b) administering the gene therapy vector to a subject, whereby the function of MS4A in the subject is modulated.

Figure 1



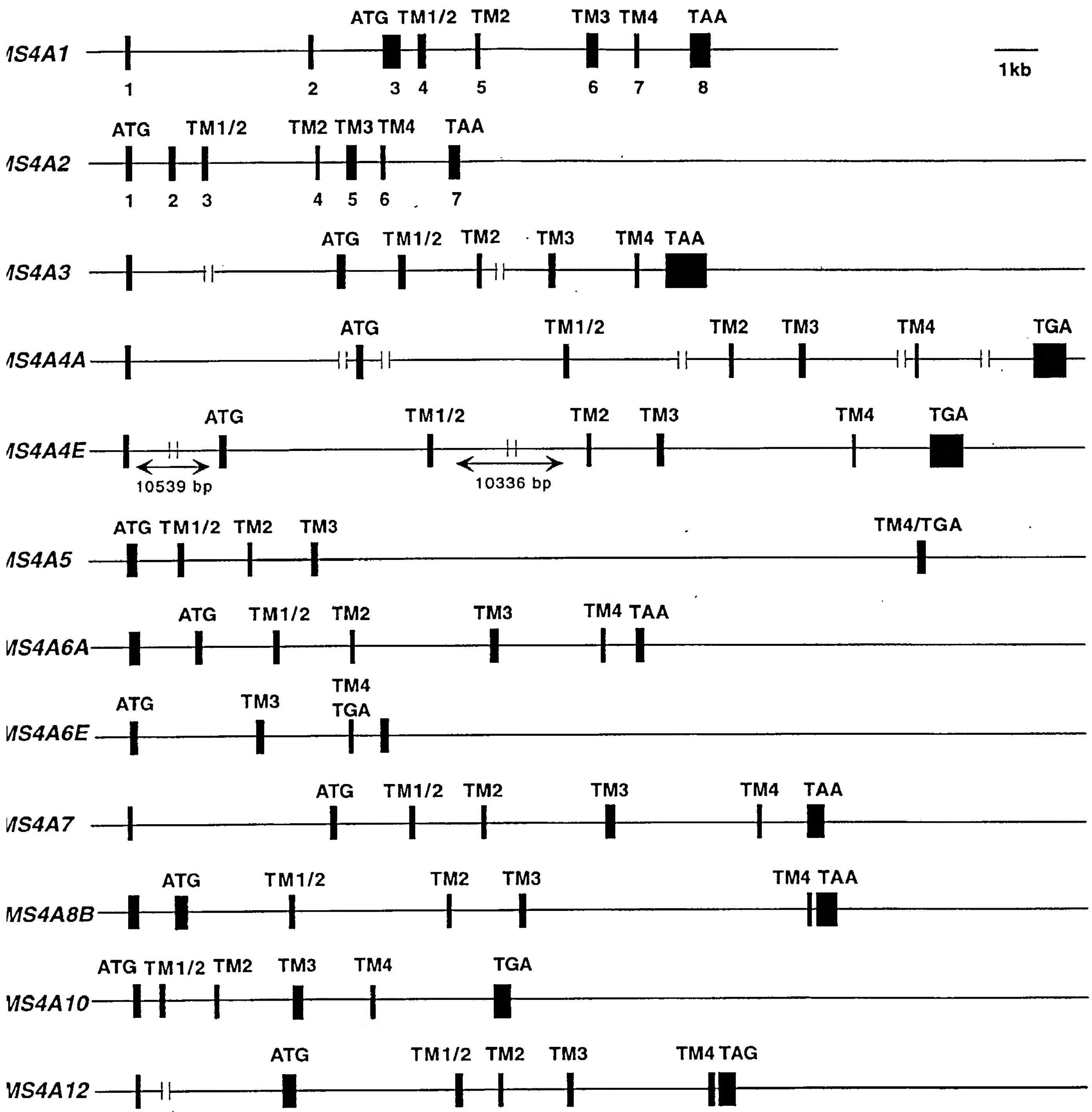

MS4A1
ATG TM1/2 TM2 TM3 TM4 TAA
1 2 3 4 5 6 7 8
1kb
MS4A2
ATG TM1/2 TM2 TM3 TM4 TAA
1 2 3 4 5 6 7
MS4A3
ATG TM1/2 TM2 TM3 TM4 TAA
MS4A4A
ATG TM1/2 TM2 TM3 TM4 TGA
MS4A4E
ATG TM1/2 TM2 TM3 TM4 TGA
10539 bp
10336 bp
MS4A5
ATG TM1/2 TM2 TM3 TM4/TGA
MS4A6A
ATG TM1/2 TM2 TM3 TM4 TAA
MS4A6E
ATG TM3 TM4 TGA
MS4A7
ATG TM1/2 TM2 TM3 TM4 TAA
MS4A8B
ATG TM1/2 TM2 TM3 TM4 TAA
MS4A10
ATG TM1/2 TM2 TM3 TM4 TGA
MS4A12
ATG TM1/2 TM2 TM3 TM4 TAG

Figure 2

Figure 3

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A4E  ATGCGGGAAA TG  GGGGCT GTAGGAGAAT GGTGTCTGAG AGAAGACAGA AAC             TGTTAGT
A4A  ATGTGGGCAA TGGTGGGGAA GTGGGGGAAT AGTGTCTGAG AGAAGATAGA GACTATAAAA TGCTGTTCAT

A4E GTGTGAGAAG ATTAGATGCT GTGGTGGGTT TGTTGCTATT CTTGTAAGGT GGTCATGGCC GGGGGAGTGG AAC  3
A4A GTGTGAGAAG ATTAGATGAT GTGGTGGGTT TGTTTTTGTT CTTACAAGGT GGTCA   CA AGAGGGGTGG AAC  3

A4E  ATT CCT GCA AAC GGG CTC AAC ATA TGC AAA TGT CTC AAT ATA GGA ATG AAA TTA     CTT  60
A4A  ATT CCT GCA AAT GGT TTC AAT ATA TGC AGA TGT CTC GAT ATA GGA ATG AAA TTA CGT CTT  63

A4E  TAG AAC AAC TTA AAT AAG TCC AAT ATA CTT GGG GCT TTA AAA AAT AAA AAG GAG AAA TTC  120
A4A  TGG AAC AAC TTA AAT AAG TCA AAT ATA CTT GGA GCT TTA AA  AAT TAA AAG GAG AGA GAT  122

A4E                              M   T   T   M   Q   G   M   E   Q   T   T   P   G
A4E  AAG| AGC ACC TTT TCT GCT GCC ATG ACA ACC ATG CAA GGA ATG GAA CAG ACC ACT CCA GGG  180
A4A  TCG AG|C ACC TTT TCT GCT GCC ATG ACA ACC ATG CAA GGA ATG GAA CAG GCC ATG CCA GGG  182

A4E   P   G   P   D   V   P   Q   L   G   N   I   D   V   I   H   S   Y   L   C   K
A4E  CCT GGC CCT GAT GTG CCC CAG CTG GGA AAC ATA GAT GTC ATA CAT TCA TAT CTG TGT AAA  240
A4A  GCT GGC CCT GGT GTG CCC CAG CTG GGA AAC ATG GCT GTC ATA CAT TCA CAT CTG TGG AAA  142

A4E   G   L   Q   E   K   F   F   K   R   K   P   K   V   L   G   V   V   R   I   L
A4E  GGA TTG CAA GAG AAG TTC TTC AAG AGG AAA CCC AAA GTC CTT GGG|GTT GTG CGG ATT CTG  300
A4A  GGA TTG CAA GAG AAG TTC TTG AAG GGA GAA CCC AAA GTC CTT GGG|GTT GTG CAG ATT CTG  302

A4E   I   A   L   M   S   L   S   M   G   I   I   M   M   C   V   A   F   S   S   Y
A4E  ATT GCC TTG ATG AGC CTT AGC ATG GGA ATA ATA ATG ATG TGT GTT GCA TTT AGT TCT TAT  360
A4A  ACT GCC CTG ATG AGC CTT AGC ATG GGA ATA ACA ATG ATG TGT ATG GCA TCT AAT ACT TAT  362

A4E   E   E   H   P   I   F   V   Y   V   A   Y   T   I   W   G   S   V   M   F   I
A4E  GAA GAA CAT CCC ATT TTT GTG TAT GTT GCG TAC ACA ATT TGG GGG TCA GTG ATG|TTT ATT  420
A4A  GGA AGT AAC CCT ATT TCC GTG TAT ATC GGG TAC ACA ATT TGG GGG TCA GTA ATG|TTT ATT  422

A4E   I   S   V   S   L   S   V   A   A   G   I   R   T   T   K   G   L   V   G   G
A4E  ATT TCA GTA TCC TTA TCA GTT GCA GCA GGA ATT AGA ACA ACA AAA GGT CTG|GTT GGA GGT  480
A4A  ATT TCA GGA TCC TTG TCA ATT GCA GCA GGA ATT AGA ACT ACA AAA GGC CTG|GTC CGA GGT  482

A4E   S   L   G   K   N   I   T   S   S   V   L   A   I   S   G   I   L   I   N   A
A4E  AGT CTA GGA AAG AAT ATC ACC AGT TCA GTC TTG GCT ATA TCA GGG ATC TTA ATC AAT GCA  540
A4A  AGT CTA GGA ATG AAT ATC ACC AGC TCT GTA CTG GCT GCA TCA GGG ATC TTA ATC AAC ACA  542

A4E   I   S   L   T   F   Y   S   F   R   Y   H   Y   C   N   H   D   Q   L   S   S
A4E  ATA AGC TTG ACG TTT TAT TCA TTC CGT TAC CAT TAC TGT AAC CAC GAT CAG TTG TCA AGT  600
A4A  TTT AGC TTG GCG TTT TAT TCA TTC CAT CAC CCT TAC TGT AAC TAC TAT GGC AAC TCA AAT  602

A4E   N   C   Y   M   T   M   S   I   L   M   G   T   D   G   M   V   L   L   L   S
A4E  AAT TGT TAC ATG ACT ATG TCC ATT TTA ATG|GGT ACG GAT GGC ATG GTG CTC CTC TTA AGT  660
A4A  AAT TGT CAT GGG ACT ATG TCC ATC TTA ATG|GGT CTG GAT GGC ATG GTG CTC CTC TTA AGT  662

A4E   V   L   E   F   C   I   A   V   S   L   S   A   F   G   C   K   V   L   C   C
A4E  GTG CTG GAA TTC TGC ATT GCT GTG TCC CTC TCT GCC TTT GGA TGT AAA GTG CTT TGT TGT  720
A4A  GTG CTG GAA TTC TGC ATT GCT GTG TCC CTC TCT GCC TTT GGA TGT AAA GTG CTC TGT TGT  722

A4E   S   P   S   E   F   V   L   I   T   P   S   N   S   H   M   A   E   I   A   S
A4E  AGC CCC AGT GAG|TTT GTG CTA ATT ACA CCA TCA AAT TCT CAC ATG GCA GAA ATA GCA TCT  780
A4A  ACC CCT GGT GGG|GTT GTG TTA ATT CTG CCA TCA CAT TCT CAC ATG GCA GAA ACA GCA TCT  782

A4E   P   T   P   L   K   T   V   *
A4E  CCC ACA CCA CTT AAG ACA GTT TGA TGCCACCAAA AGATTAACAG AAGAATGCTC CAGAAATCTA      844
A4A  CCC ACA CCA CTT AAT GAG GTT TGA GGCCACCAAA AGATCAACAG ACAAATGCTC CAGAAATCTA      846

A4E  TGCTGACTGT AACACAAGAA CC CACATGA GAAAGTACCA GAATCCAACT CCAATACTGA TAGACATATT    913
A4A  TGCTGACTGT GACACAAGAG CCTCACATGA GAAATTACCA GTATCCAACT TCGATACTGA TAGACTTGTT    916

A4E  GATATCATTA TTATATGGAA TCCAATTATG ACCTCTGTGT GTGTGTGTGT GTGTATATAT ATATATACAT    983
A4A  GATATTATTA TTATATGTAA TCCAATTATG AACTGTGTGT GTATA                               961

A4E  ATATATATAT ATACATATAT ATATATGTGT GTGTGTGTAT ATATTCAAAA TTTTGTTCTC ATTTTTTCCC    1053
A4A                                  GAGAGATAAT AAATTCAAAA TTATGTTCTC ATTTTTTTCC    1001

A4E  CTGGAACTCA ACAACTAATT TCATTGGCCC TTTATCGAGA GTACTAGAAG TTAAATTAAT AAATAATGCA    1123
A4A  CTGGAACTCA ATAACTCATT TCACTGGCTC TTTATCGAGA GTACTAGAAG TTAAATTAAT AAATAATGCA    1071

A4E  TTTAATGAGG CAGCAGCACT TGAAAGGTTT TCATTCATCA TTAGGACTTT ATATAAAGGC ATTAAACTGGC   1194
A4A  TTTAATGAGG CAACAGCACT TGAAAGTTTT TCATTCATCA TAAGAACTTT ATATAAAGGC ATTACATTGGC   1142

A4E  AAATAAGATT TGGAAGCAGA AGGGCAAAA     GGTATTG CTAAAACGAG GTCTCCATGC AAA CACATA    1259
A4A  AAATAAGGTT TGGAAGCAGA AGAGCAAAAA AAAGATATTG TTAAAATGAG GCCTCCATGC AAAACACATA    1212

A4E  CTTCTGCTCC CCTGTATAAC ATTCCTCTCA CTTACTTGAC TTTTTTTCTG CCATATTTGG GGACCAAAGT    1329
A4A  CTTCC CTCC          C ATTTAT TTA    ACTT    TTTTTTTCTC CTACCTATGG GGACCAAAGT    1265

A4E  GCTTTTTCCT TCATGAAGTG GAGATTCATG CCCTTCTCCC CCCTCCTTTT  CCTTCT    GCTTTCCTTC    1395
A4A  GCTTTTTCCT TCAGGAAGTG GAGATGCATG GCCATCTCCC CCTCCCTTTT TCCTTCTCCT GCTTTTCTTT    1335

A4E  ACCCATAGAA AGTACCTTGG AATAGTATAG TCAGTCCTTG CATGTGCACA AGCTATCATT TCAGTAAAGG    1465
A4A  CCCCATAGAA AGTACCTTGA AGTAGCACAG TCCGTCCTTG CATGTGCACG AGCTATCATT TGAGTAAAAG    1405

A4E  TATACATGGA GTAAAAATCA TATGAAGGAT CAGATTCAAC TTATATTTTC TATTTTTTCT TCTTCCTCTC    1535
A4A  TATACATGGA GTAAAAATCA TATTAAGCAT CAGATTCAAC TTATATTTTC TATTTCATCT TCTTCCTTTC    1475

A4E  CCTTCCCCCA CCTTCTGCTG GGCAGAATTA TATCTTAATC AAATGTGTAT CCTGTGTCAC ATATGGAAAT    1605
A4A  CCTTCTCCCA CCTTCTACTG GGCATAATTA TATCTTAATC ATATATGGAA  ATGTGCAAC ATATGGTATT    1544

A4E  GTGCAACATA TGGTATTTGT TAATGTTTGT TAATTACATT TGCTTTTTTA TTGCAGAGCA AAAATAAAAT    1675
A4A                            TGT TAAATACG   TTTGTTTTTA TTGCAGAGCA AAAATAAATC    1585

A4E      TAGAAG CAATACTTTC ATGT                                                      1695
A4A  AAATTAGAAG CAATAAAAAA AAAAAAAAAA AAAA                                           1619
```

Figure 4

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A6E                        M   T   S   Q   P   I   S   N   E   T   I   I   M
A6E          TT GGC AAC ACC ATT ATG ACA TCA CAA CCT ATT TCC AAT GAG ACC ATC ATA ATG   53
A6A          TT GGC AAC ACC ATC ATG ACA TCA CAA CCT GTT CCC AAT GAG ACC ATC ATA GTG  277

A6E   L   P   S   N   V   I   N   F   S   Q   A   E   K   P   E   P   T   N   Q   G
A6E  CTC CCA TCA AAT GTC ATC AAC TTC TCC CAA GCA GAG AAA CCC GAA CCC ACC AAC CAG GGG  113
A6A  CTC CCA TCA AAT GTC ATC AAC TTC TCC CAA GCA GAG AAA CCC GAA CCC ACC AAC CAG GGG  337

A6E   Q   D   S   L   K   K   R   L   Q   A   K   V   K   V   I   G
A6E  CAG GAT AGC CTG AAG AAA CGT CTA CAG GCA AAA GTC AAA GTT ATT GGG|                 161
A6A  CAG GAT AGC CTG AAG AAA CAT CTA CAC GCA GAA ATT AAA GTT ATT GGG| (exons MS4A6A)  397

A6E   V   H   S   S   L   A   G   S   I   L   S   A   L   S   A   L   V   G   F   I
A6E  GTG CAT AGC AGC CTG GCT GGA AGC ATT CTG AGT GCT CTG TCT GCC CTG GTG GGT TTC ATT  221
A6A  GTG CAT AGC AGC CTG GTT GGA AGC ATT CTG AGT GCT CTG TCT GCC CTG GTG GGT TTC ATT  637

A6E   L   L   S   V   N   P   A   A   L   N   P   A   S   L   Q   C   K   L   D   E
A6E  CTC CTG TCT GTC AAC CCG GCT GCA TTA AAT CCT GCC TCA TTG CAG TGT AAG TTG GAC GAA  281
A6A  ATC CTG TCT GTC AAA CAG GCC ACC TTA AAT CCT GCC TCA CTG CAG TGT GAG TTG GAC AAA  697

A6E   K   D   I   P   T   R   L   L   L   S   Y   D   Y   H       S   P   Y   T   M
A6E  AAG GAT ATA CCA ACC AGA CTT CTT CTT TCT TAT GAT TAT CAT     TCA CCT TAC ACC ATG  338
A6A  AAT AAT ATA CCA ACA AGA AGT TAT GTT TCT TAC TTT TAT CAT GAT TCA CTT TAT ACC ACG  757

A6E   D   C   H   R   A   K   A   S   L   A   G   T   L   S   L   M   L   V   S   T
A6E  GAC TGC CAT AGA GCC AAA GCC AGT CTG GCT|GGA ACT CTG TCT CTG ATG CTG GTT TCT ACT  398
A6A  GAC TGC TAT ACA GCC AAA GCC AGT CTG GCT|GGA ACT CTC TCT CTG ATG CTG ATT TGC ACT  817

A6E   V   L   E   F   C   L   A   V   L   T   A   V   L   Q   W   K   Q   T   V   *
A6E  GTG TTG GAG TTC TGC CTA GCT GTG CTC ACT GCT GTG CTG CAG TGG AAA CAG ACT GTC TGA  458
A6A  CTG CTG GAA TTC TGC CTA GCT GTG CTC ACT GCT GTG CTG CGG TGG AAA CAG GCT TAC TCT  877

A6E    C TTC CCT  GG|                                            TTA CTC ATG TCC  479
A6A  GAC TTC CCT GGG|AGT GTA CTT TTC CTG CCT CAC AGT TAC ATT GGT AAT TCT GGC ATG TCC  937

A6E  TCA AAG ACG ACT CAT GAT GCT GGA TAT GAA GAA CTA TTG ACT TCT TGG GAA AAA ACG GAG  539
A6A  TCA AAA ATG ACT CAT GAC TGT GGA TAT GAA GAA CTA TTG ACT TCT TAA GAA AAA AGG GAG  997

A6E  AAA TAT TAA TTG GAA AGT TGA TTT TTA TGA TAA TAT GGA AAA CCT AAC CAT TAT AAA AAA  599
A6A  AAA TAT TAA TCA GAA AGT TGA TTC TTA TGA TAA TAT GGA AAA GTT AAC CAT TAT AGA AAA 1057

A6E  GCA AA  CTT GAG TTT CCT AAA TGT AAG CAT TTA AAG TAA ATG CAT ATT TGT TTT AAA AAA  658
A6A  GCA AAG CTT GAG TTT CCT AAA TGT AAG CTT TTA AAG TAA  TG AAC ATT  AA AAA AAA CCA 1115

A6E  TTA                                                                             661
A6A  TTA TTT CAC TGT CA                                                             1129
```

Figure 5

```
A10                                                 C CAG GGC CCC CAT CCA GCA TCA    22
a10                                                 C CAG GGT TCC CGG CCA GGG TCA    74

A10   M   K   A   E       A   T   V   I   P   S   R   C   A   R   G   L   P   S   W
A10  ATG AAA GCA GAA     GCC ACA GTT ATT CCC AGC CGT TGT GCT AGG GGG CTC CCA TCA TGG    79
a10  ATG GCT GGC CAA GCT CCC ACA GCG GTT CCC GGC AGT GTT ACT GGA GAA GTC TCA CGA TGG   134

A10   Q   V   L   S   P   V   Q   P   W   Q   T   S   A   P   Q   N   T   T   Q   P
A10  CAA GTC CTC AGC CCA GTC CAG CCC TGG CAG ACA AGT GCA CCC CAG AAC ACG ACC CAG CCC   139
a10  CAG AAC CTA GGA CCT GCC CAG CCT GCA CAG AAA GTA                         GCC CAG CCC   179

A10   K   L   L   A   P   H   Q   H       E   K   S   Q   K   K   S   S   L   L   K
A10  AAG CTC CTG GCT CCA CAC CAG CAC     GAG AAG TCC CAG AAG AAG AGC AGC CTT CTT AAG   196
a10  CAA AAC CTG GTT CCA GAT GGG CAC CTT GAG AAA GCC CTG GAG GGA AGT GAC CTT CTA CAG   239

A10   E   L   G   A   F   H   I   T   I   A   L   L   H   L   V   F   G   G   Y   L
A10  GAG CTG GGG|GCC TTC CAC ATC ACC ATC GCT CTG CTG CAC CTG GTC TTT GGG GGC TAC CTG   256
a10  AAG TTG GGT GGC TTT CAC ATC GCC ATT GCT TTT GCA CAC TTG GCC TTT GGT GGC TAC TTG   299

A10   A   S   I   V   K   N   L   H   L   V   V   L   K   S   W   Y   P   F   W   G
A10  GCC TCT ATA GTC AAG AAC CTT CAC CTG GTG GTG CTG AAG TCT TGG TAT CCA TTC TGG GGG   316
a10  ATC TCC ACA GTC AAG AAT CTG CAC CTG GTG GTG TTG AAG TGC TGG TAT CCA CTC TGG GGA   359

A10   A   A   S   F   L   I   S   G   I   L   A   I   T   M   K   T   F   S   K   T
A10  GCT GCC TCT|TTT CTC ATT TCA GGG ATC TTG GCG ATA ACA ATG AAG ACC TTT TCT AAA ACT   376
a10  ACT GTC TCT TTT CTC GTT GCA GGG ATG GCG GCC ATG ACA ACA GTG ACA TTC CCT AAG ACC   419

A10   Y   L   K   M   L   C   L   M   T   N   L   I   S   L   F   C   V   L   S   G
A10  TAC CTG|AAG ATG TTG TGC CTG ATG ACA AAC CTC ATC AGC CTC TTT TGC GTG CTG TCT GGC   436
a10  TCT CTG AAG GTT CTC TGT GTG ATA GCC AAC GTT ATC AGC TTG TTC TGC GCA CTG GCC GGC   479

A10   L   F   V   I   S   K   D   L   F   L   E   S   P   F   E   S   P   I   W   R
A10  CTC TTC GTC ATC TCC AAG GAT CTC TTT CTG GAG AGC CCA TTT GAG TCC CCG ATC TGG AGA   496
a10  TTC TTT GTC ATT GCC AAG GAC CTC TTC CTG GAG GGT CCT TTT CCA TGG CCA ATC TGG AGA   539

A10   M   Y   P   N   S   T   V   H   I   Q   R   L   E   L   A   L   L   C   F   T
A10  ATG TAC CCC AAC TCC ACG|GTC CAC ATC CAG AGG CTG GAG CTG GCC TTG CTC TGC TTC ACT   556
a10  CCA TAC CCT GAA CCC ACA ACC TAC ATC CAA AGG CTA GAG CTG ACC CTG TTC TGC TTC ACC   599

A10   V   L   E   L   F   L   P   V   P   T   A   V   T   A   W   R   G   D   C   P
A10  GTC CTA GAG CTC TTC CTG CCA GTG CCC ACA GCT GTC ACA GCC TGG AGA GGG GAC TGC CCA   616
a10  TTC CTG GAG ATC TTC CTG TCG GGG TCC ACA GCC ATC ACA GCC TAT AGG ATG AAA CGC CTG   659

A10   S   A   K       N   D   D   A   C   L   V   P   N   T   P   L   H   L   K   G
A10  TCT GCA AAG     |AAT GAT GAT GCA TGC CTT GTT CCG AAT ACA CCA TTG CAT CTC AAA GGC   673
a10  CAA GCA GAG GAC AAG GAT GAC ACC CCT TTC GTT CCT GAC ACA CCT ATG GAA CTT AAA GGT   719

A10   L   P   V   E   P   P   P   S   Y   Q   S   V   I   Q   G   D   A   Q   H   K
A10  CTG CCG GTG GAG CCC CCG CCA TCC TAC CAG AGT GTG ATT CAA GGC GAC GCA CAA CAC AAG   733
a10  CTA TCG CTG GGG CCA CCA CCA TCC TAT AAA GAT GTG GCC CAA GGA CAC TCC TCC TCT GAT   779

A10   Q   H   Q   R   *
A10  CAA CAT CAG AGG TGA AGA GGT TTG GCC CTG GCT CCC CAG ACC TTC AGA ACC CAC GCT TGG   793
a10  ACT GGC AGA GCC TTG GCC ACC AGC TCT GGA CTG CTG CTG GCC TCT GAC TCC TTC CAT CAA   839


A10  CCC CTT CTC CCT GGC ATA GAT AGA AAC CAG TTC CCA GGG GAGCTGTAAG GGGCAAGGGG          852
a10  GCT CTG CTT CAC ACG GGC CCA AGG ACC TTG AGA AAG TAG TCTAAGACTA CTAGACTTTC          898

A10  CTAACAGTGC TGCAGCTAGA ACTGCCTGGC CTGGGAGTGG TAGCCAAGGC AGCAATGGTC CTATCCCCTC    922
a10  CCAATAAAGA CTGAGTTCCT GCAATGCCAA GTCCTTCCCT GCCTCCCATT GGCTGGACTC CGGTGATGAC    968

A10  AGGCACTAGA GGAACTCCCA GGGCCCCCCA GGAACATCTC AGCACCTGAC                          972
a10  ACAGGCGTGA CTTGCACAAA TAAATGCATA ACCACTATGA AAAAAAAAAA                         1018
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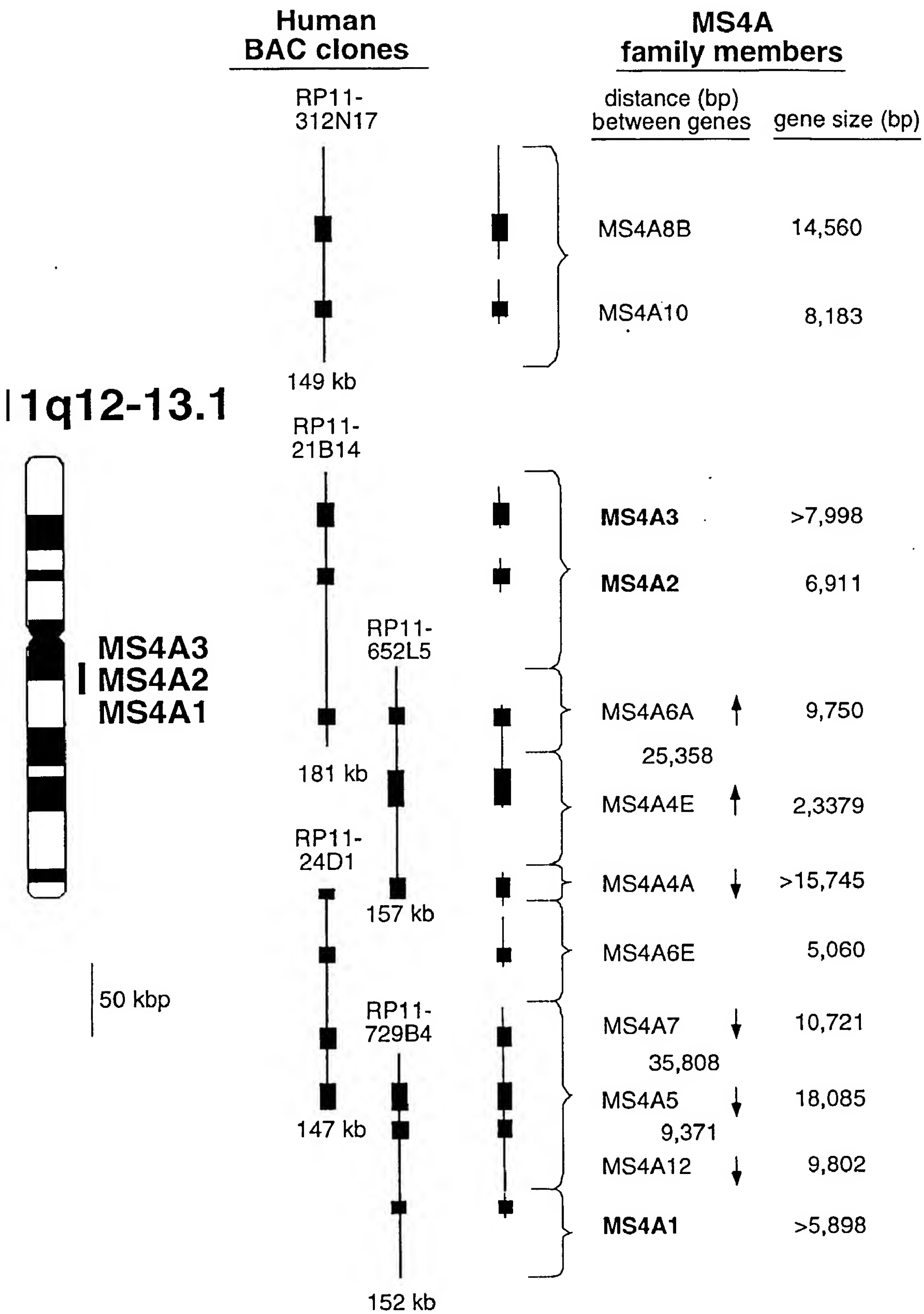
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Figure 6

Figure 7

```
                                                                                              [------------TM1-----------
A1                                MTTPRNSVNGTFPAEPMKGPIAMQSGPKPLFRRMSSLVGPTQSFFMRESKTLG|AVQIMNGLFHIALGGLLMIPAGI   76
a1                                        MSGPFPAEPTKGPLAMQPAPKVNLKRTSSLVGPTQSFFMRESKALG AVQIMNGLFHITLGGLLMIPTGV   69
A2                      MDTESNRRANLALP  QEPS|SVPAFEVLEISPQEVSSGRLLKSASSPPLHTWLTVLKKEQEFLG|VTQILTAMICLCFGTVVCSVLDI   85
a2                      MDTENRSRADLALPNPQESS SAPDIELLEASP          AKAAPPKQTWRTFLKKELEFLG ATQILVGLICLCFGTIVCSVLYV   77
A3                                 MASHEVDNAELGSASAHGTPGSETGPEELNTSVYHPINGSPDYQKAKLQVLG AIQILNAAMILALGVFLGSLQYP   75
a3                                                       MKPEETGGSVYQPLDESRHVQRGVLQALG AIQILNGILILALGIFLVCLQHV   52
A4A                                   MTTMQGMEQAMPGAGPGVPQLGNMAVIHSHLWKGLQEKFLKGEPKVLG|VVQILTALMSLSMGITMMCMASN   71
a4B                                     MQGQEQTTMAVVPGVAVPSKNSVMTSQMWNEKKEKFLKGEPKVLG VLQVMIAIINLSLGIIILTTLF   67
a4C                                     MQGQEQTTMAVVPGGAPPSENSVMKSQMWNENKEKFLKGEPKVLG VVQVMIALINLSFGIIILANLS   67
a4D                                     MQGLAQTTMAVVPGGAPPSENSVIKSQMWNKNKEKFLKGEPKVLG AIQVMIAFINFSLGIIIILNRV   67
A4E                                   MTTMQGMEQTTPGPGPDVPQLGNIDVIHSYLCKGLQEKFFKRKPKVLG|VVRILIALMSLSMGIIMMCVAFS   71
A5                                 MDSSTAHSPVFLVFPPEITASEYESTELSATTFSTQSPLQKLFARKMKILG|TIQILFGIMTFSFGVIFLFTLLK   74
A6A                                  MTSQPVPNETIIVLPSNVINFSQAEKPEPTNQGQDSLKKHLHAEIKVIG|TIQILCGMMVLSLGIILASASFS   72
a6B                                  MIPQVVTSETVAMISPNGMSLPQTDKPQPFHQWQDSLKKHLKAEIKVMA AIQIMCAVMVLSLGIILASVPSN   72
a6C                                  MIPQVVTNETITTISPNGINFPQKDESQPTQQRQDSLKKHLKAEIKVIV AIQIMCAVTVLALGIILASVPPV   72
a6D                                  MIPQVVTSETVTVISPNGISFPQTDKPQPSHQSQDSLKKHLKAEIKVMA AIQIMCAVMVLSLGILIASVPSN   72
A6E                                  MTSQPISNETIIMLPSNVINFSQAEKPEPTNQGQDSLKKRLQAKVKVIG|
A7                                   MLLQSQTMGVSHSFTPKGITIPQREKPGHMYQNEDYLQNGLPTETTVLG|TVQILCCLLISSLGAILVFAPYP   72
a7                                   MRLQLGTKNIGWDCFPKDIIHKREKTGHTYEKEDDLLIGVPSEATLLG TIQLLCALILASFGGILVSASY   71
A8B                MNSMTSAVPVANSVLVVAPHNGYPVTPGIMSHVPLYPNSQPQVHLVPGNPPSLVSNVNGQPVQKALKEGKTLG|AIQIIIGLAHIGLGSIMATVLVG   96
a8B    MEPEQERLTWQPGTVSMNTVTSPGPMANSVYVVAPPNSYPVVPGTVPQMPIYPSNQPQVHVISGHLPGLVPAMTEPPAQRVLKKGQVLG AIQILIGLVHIGLGSIMITNLFS 112
A10                          MKAEA TVIPSRCARGLPSWQVLSPVQPWQTSAPQNTTQPKLLAPHQH EKSQKKSSLLKELG|AFHITIALLHLVFGGYLASIVKN   84
a10                          MAGQAPTAVPGSVTGEVSRWQNLGPAQPAQKVA     QPQNLVPDGHLEKALEGSDLLQKLG GFHIAIAFAHLAFGGYLISTVKN   81
A12 MMSSKPTSHAEVNETIPNPYPPGSFMAPGFQQPLGSINLENQAQGAQRAQPYGITSPGIFASSQPGQGNIQMINPSVGTAVMNFKEEAKALG|VIQIMVGLMHIGFGIVLCLISFS 115
a12 MMSSKPTTYPGVYGTTPDLYPPSNFMVPGSQQPPGFINPRIQVQSSQ   APFIVSPGIPNNSQQVQGNIQMVNPGTGKAATNFKEEAKTLG AIQILIGSMHIGFGIILGLMGRT 112

    --------TM2----------------------]                 [----------TM3----------]
A1        YAPICVTV   WYPLWGGIM|YIISGSLLAA TEKNSRKCL|VKGKMIMNSLSLFAAISGMILSIMDILNIKISHFLKMESLNFIRAHTPYINIYNCEPANPSEKNSPSTQY 182
a1        FAPICLSV   WYPLWGGIM YIISGSLLAAAAEKTSRKSL VKAKVIMSSLSLFAAISGIILSIMDILNMTLSHFLKMRRLELIQTSKPYVDIYDCEPSNSSEKNSPSTQY 176
A2  SHIEGDIFSSFKA    GYPFWGAIF|FSISGMLSIISERRNAT YL VRGSLGANTASSIAGGTGITILIINLKKSL                 AYIHIHSCQKFFETK 171
a2   SDFDEEVLLLYKL GYPFWGAVL FVLSGFLSIISERKN TLYL VRGSLGANIVSSIAAGTGIAMLILNLTNNF                 AYMNNCKNVTEDDG 162
A3  YHFQKHFFFFTFYT GYPIWGAVF FCSSGTLSVVAGIKP TRTW IQNSFGMNIASATIALVGTAFLSLNIAVNI                 QSLRSCHSSSESPDL 162
a3  SHHFRHFFFFTFYT GYPLWGAVF FISSGSLTVAAGRNP TRML MQNSFGINIASTTIAFVGTVFLSVHLAFN*
A4A TYGSNPISVYI    GYTIWGSVM|FIISGSLSIAAGIRT TKGL|VRGSLGMNITSSVLAASGILINTFSLAFYS              FHHPYCNYYGNSNN 154
a4B    SELPTSVML   MVPIWGSIM|FIVSGSLSIAAGVTP TKCL|IVASLTLNTITSVLAATASIMGVVSVAVGS                         QFP 137
a4C    SEPLISVVL   MAPIWGPIM FIVSGSLSIAAGVKP TKSL|IISSLTLNTITSVLAATASIMGVVSVAVGS                         QFP 137
a4D    SERFMSVLL   LAPFWGSIM FIFSGSLSIAAGVKP TKAM IISSLSVNTISSVLAVAASIIGVISVISGV                          FR 136
A4E SYEEHPIFVYV    AYTIWGSVM|FIISVSLSVAAGIRT TKGL|VGGSLGKNITSSVLAISGILINAISLTFYS              FRYHYCNHDQLSSN 154
A5    PYPRFPPFIFLS GYPFWGSVL|FINSGAFLIAVKRKT TETL|IILSRIMNFLSALGAIAGIILLTFGFIL                DQNYICGYSHQNSQ 155
A6A PNFTQVTSTLLNS  AYPFIGPFF|FIISGSLSIATEKRL TKLL|VHSSLVGSILSALSALVGFIILSVKQATLNPASLQ     CELDKNNIPTRSYVSYFYHDSLYTTD 174
a6B LHFTSVFSVLLKS  GYPFIGALF FIVSGILSIVTETKS TKIL|VDSSLTLNILSVSFAFMGIIIISVSLAGLHPASEQ      CLQSKELRPTEYHYYQ   FLDRNE 170
a6C PYFNSVFSVLLKS  GYPFIGALF|FIASGILSIITERKS TKPL VDASLTLNILSVSFAFVGIIIISVSLAGLHPASEQ      CKQSKELSLIEHDYYQPFYNSDRSE 173
a6D LHFTSVFSILLES  GYPFVGALF FAISGILSIVTEKKM TKPL VHSSLALSILSVLSALTGIAILSVSLAALEPALQQ      CKLAFTQLDTTQDAYHFFSPEPLNS 173
A6E                                             |VHSSLAGSILSALSALVGFILLSVNPAALNPASLQ     CKLDEKDIPTRLLLSYDYHSPYT MD 109
A7  SHFNPAISTTLMS  GYPFLGALC|FGITGSLSIISG KQSTKPF|DLSSLTSNAVSSVTAGAGLFLLADSMVALR      TASQHCGSEMDYLSSLPYSEYYYPIYEIKD 173
a7    FNPEVSTTLIS  GYLFIGSLC FAIAGILSIISEKIS TKPF ALSSLASNVASSVVAVIGLFLFTYCLIALG       SAFPHCNSEKKFLSLLSYLKSHHWKNEDKN 170
A8B    EYLSISFYG   GFPFWGGLW|FIISGSLSVAAENQPYSYCL|LSGSLGLNIVSAICSAVGVILFITDLSIPH                   PYAYPDYYPY  174
a8B    HYTPVSLYG   GFPFWGGIW FIISGSLSVAAETQPNSPCL LNGSVGLNIFSAICSAVGIMLFITDISISS                 GYIYPSYYPYQE 192
A10       LHLVVLKS WYPFWGAAS|FLISGILAITMKTFS KTYL|KMLCLMTNLISLFCVLSGLFVISKDLFLES              PFESPIWRMYPNST 164
a10       LHLVVLKC WYPLWGTVS FLVAGMAAMTTVTFP KTSL KVLCVIANVISLFCALAGFFVIAKDLFLEG              PFPWPIWRPYPEPT 161
A12 FREVLGFASTAVIG GYPFWGGLS|FIISGSLSVSASKEL SRCL|VKGSLGMNIVSSILAFIGVILLLVDMCING                     VAGQD 192
a12 YMQVLGFASLAFVS GYPFWGGLS FIITGILCILASKKS SPAL IKSSLGMSIVSSFFAFIGMILLLVDESING                     LPEQD 189

           [---------TM4------------]
A1  CYSIQSLFL|GILSVMLIFAFFQELVIAGIVENEWKRTCSRPKS| NIVLLSAEEKKEQTIEIKEEVVGLTETSSQPKNEEDIEIIPIQEEEEEETETNFPEPPQDQESSPIENDSSP297
a1  CNSIQSVFL GILSAMLISAFFQKLVTAGIVENEWKRMCTRSKS  NVVLLSAGEKNEQTIKMKEEIIELSGVSSQPKNEEEIEIIPVQEEEEEEAEINFPAPPQEQESLPVENEIAP291
A2  CFMASFS|T EIVVMMLFLTILGLGSAVSLTICGAGEELKGNK| VPEDRVYEELNIYSATYSELEDPGEMSPPIDL                                           244
a2  C FVASFTT ELVLMMLFLTILAFCSAVLFTIYRIGQELESKK  VPDDRLYEELNVYSPIYSELEDKGETSSPVDS                                           235
A3  CNYMGSISN GMVSLLLILTLLELCVTISTIAMWCNANCCNSRE  EISSPPNSV                                                                  214
A4A CHGTMSILM|GLDGMVLLLSVLEFCIAVSLSAFGCKVLCCTPGG| VVLILPSHSHMAETASPTPLNEV                                                    220
a4B FRYNYTITK|GLDVLMLIFNMLEFCLAVSVSAFGCEASCCNSRE| VLVVLPSNPVETVMAPPMTLQPLLPSEHQGTNVPGNVYKNHPGEIV                             226
a4C FRYNYTITK GLDILMLILNMLEFCIAVSISAFGCKASCCNSSE| VLVVLPSNPAVTVMAPPVTLQPLPPSEHQGKNVPENVYKNHSEEIV                             226
a4D QFRSQPAIA SLDVLMTILNMLEFCIAVSVSAFGCKASCCNSSE  VLVVLPSNSAVTVTAPPMILQPLPPSECQGKNVPENLYRNQPGEIV                             225
A4E CYMTMSILM|GTDGMVLLLSVLEFCIAVSLSAFGCKVLCCSPSE| FVLITPSNSHMAEIASPTPLKTV                                                    220
A5  CKAVTVLFL|GILITLMTFSIIELFISLPFSILGCHSEDCDCEQCC                                                                           200
A6A CYTAKASLA|GTLSLMLICTLLEFCLAVLTAVLRWKQAYSDFPG| SVLFLPHSYIGNSGMSSKMTHDCGYEELLTS                                            248
a6B CFAAKSVLA GVFSLMLISTMLELGLAVLTAMLWWKQSHSNIPG  NVMFLPHSSNNDSNMESKVLCNPSYEEQLVC                                            244
a6C CAVTKSILT GALSVMLIISVLELGLALLSAMLWLREGVLTSLRM                                                                            217
a6D CFVAKAALT GVFSLMLISSVLELGLAVLTATLWWKQSSSAFSG  NVIFLSQNSKNKSSVSSESLCNPTYENILTS                                            247
A6E CHRAKASLA|GTLSLMLVSTVLEFCLAVLTAVLQWKQTV                                                                                  147
A7  CLLTSVSLT|GVLVVMLIFTVLELLLAAYSSVFWWKQLYSNNPG|SSFSSTQSQDHIQQVKKSSSRSWI                                                    240
a7  CYLAYVGAM SALGMMLLFTVLEVFLAGYSSIFWWKQVYSNKPG  GTFFLPQSQDHTQLVKSNLLQ                                                       234
A8B  AWGV|NPGMAISGVLLVFCLLEFGIACASSHFGCQLVCCQSSN| VSVIYP NIYAANPVITPEPVTSPPSYSSEIQANK                                        250
a8B ENLGV RTGVAISSVLLIFCLLELSIASVSSHFGCQVACCHYNN  PGVVIP|NVYAANPVVIPEPPNPIPSYSEVVQDSR                                          268
A10       |VHIQRLELALLCFTVLELFLPVPTAVTAWRGDCPSAK|NDDACLVPNTPLHLKGLPVEPPPSYQSVIQGDAQHKQHQR                                    241
a10        TYIQRLELTLFCFTFLEIFLSGSTAITAYRMKRLQAEDKDDTPFVPDTPMELKGLSLGPPPSYKDVAQGHSSSDTGRALATSSGLLLASDSFHQALLHTGPRTLRK         267
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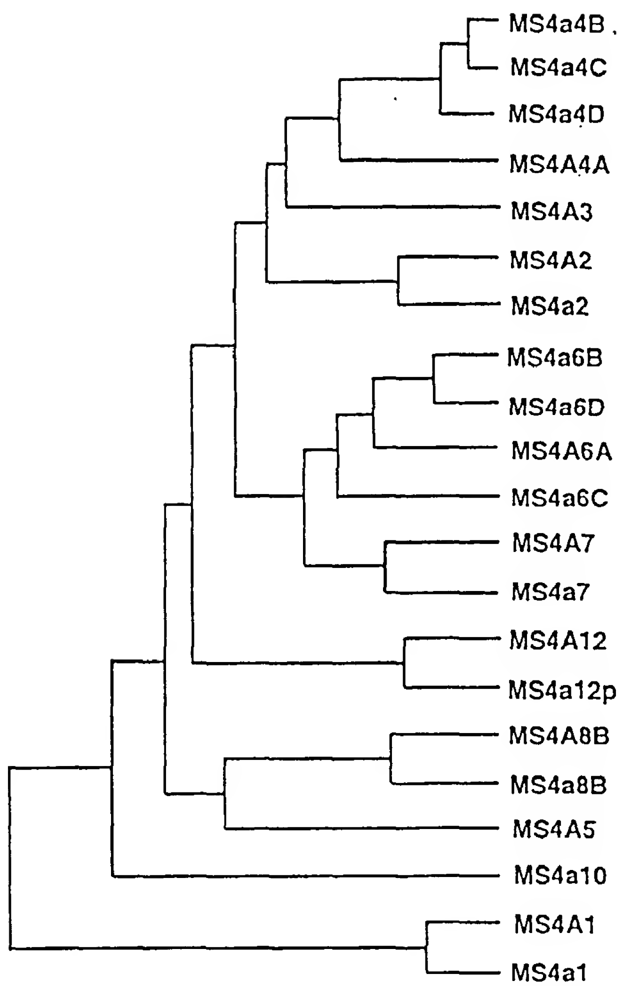
Figure 8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MS4a7
MS4A12
MS4a12p
MS4A8B
MS4a8B
MS4A5
MS4a10
MS4A1
MS4a1

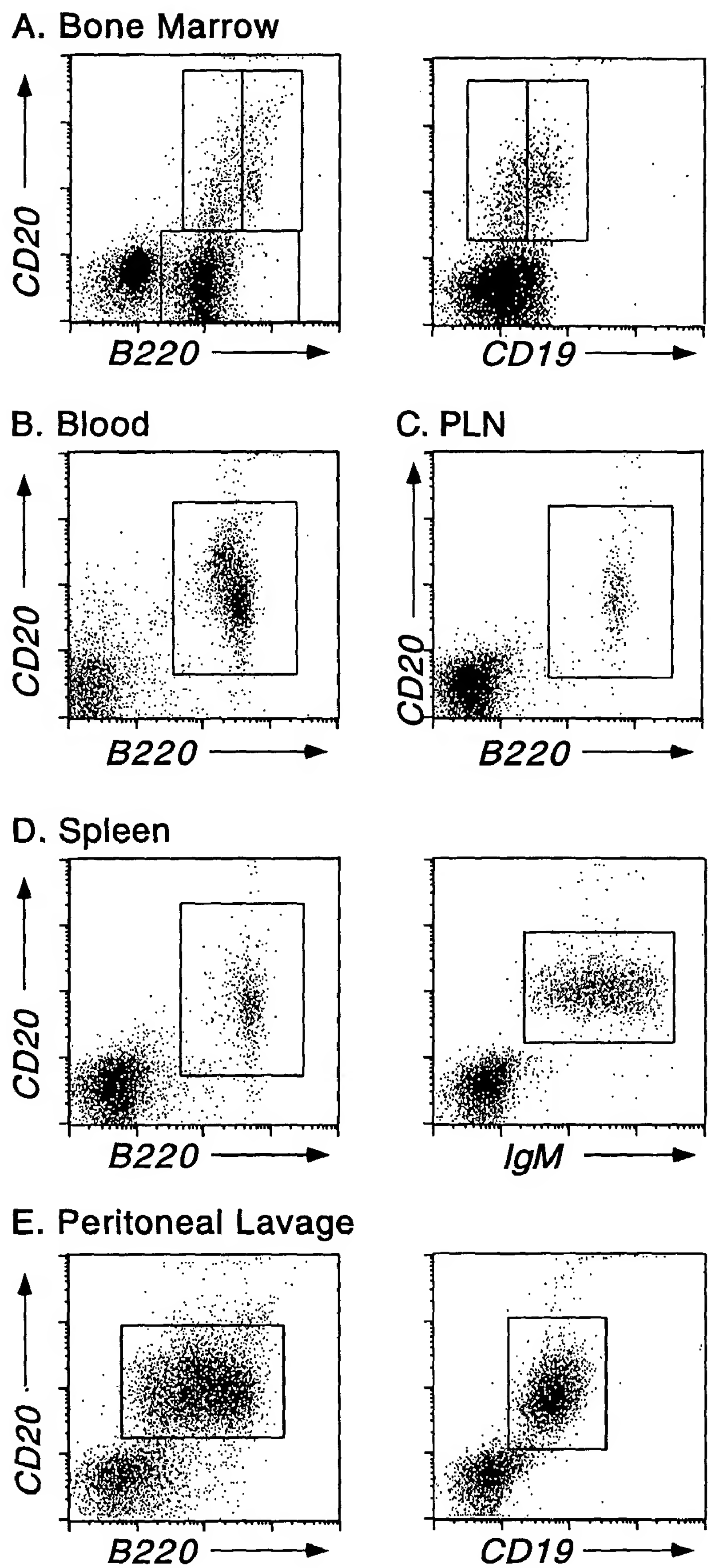
A. Bone Marrow
CD20
B220
CD19
B. Blood
CD20
B220
C. PLN
CD20
B220
D. Spleen
CD20
B220
IgM
E. Peritoneal Lavage
CD20
B220
CD19

Figure 9

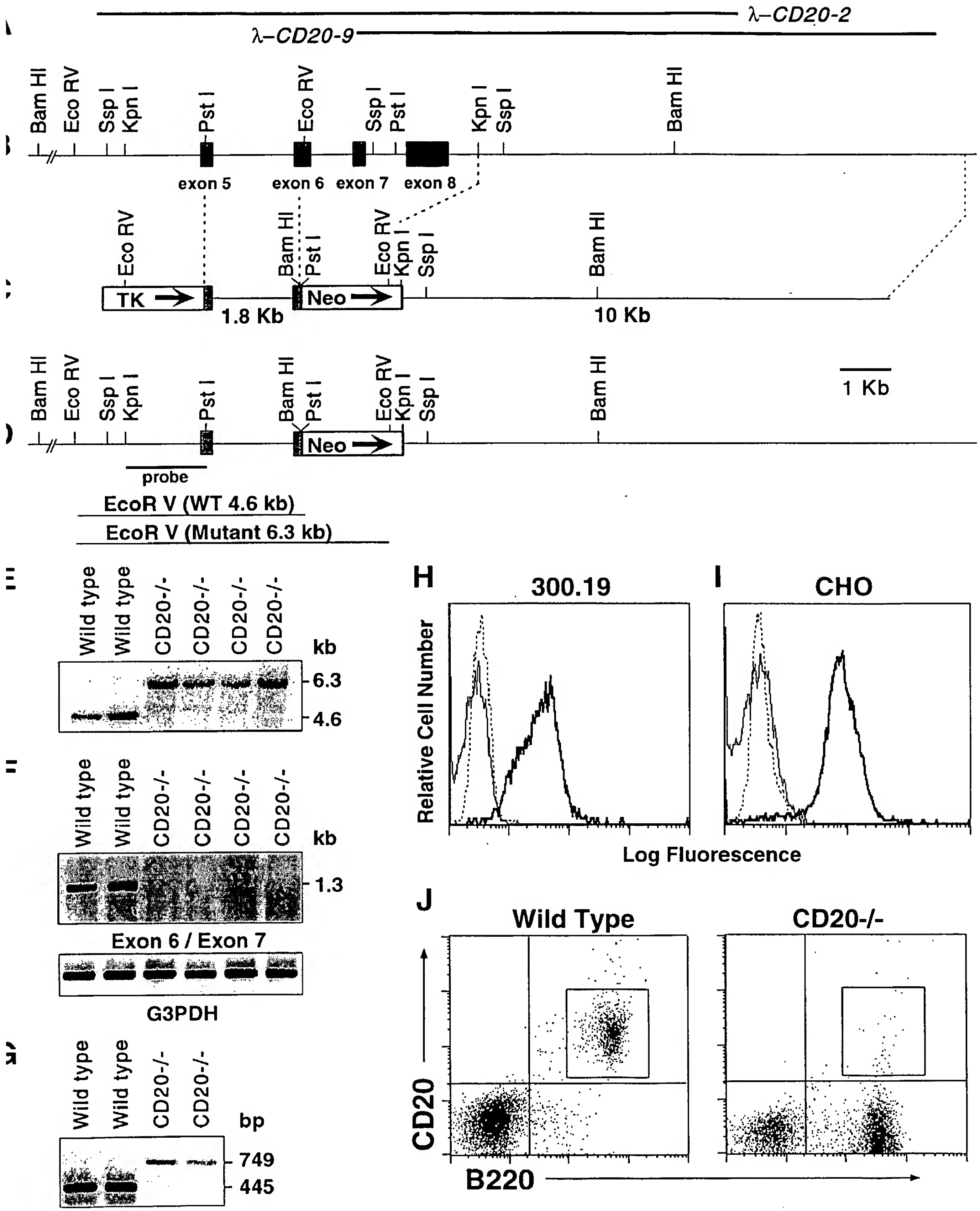
λ-CD20-2
λ-CD20-9
Bam HI
Eco RV
Ssp I
Kpn I
Pst I
exon 5
exon 6
exon 7
exon 8
TK
Neo
1.8 Kb
10 Kb
1 Kb
probe
EcoR V (WT 4.6 kb)
EcoR V (Mutant 6.3 kb)
Wild type
CD20-/-
kb
6.3
4.6
1.3
Exon 6 / Exon 7
G3PDH
bp
749
445
H
300.19
I
CHO
Relative Cell Number
Log Fluorescence
J
Wild Type
CD20-/-
CD20
B220

Figure 10

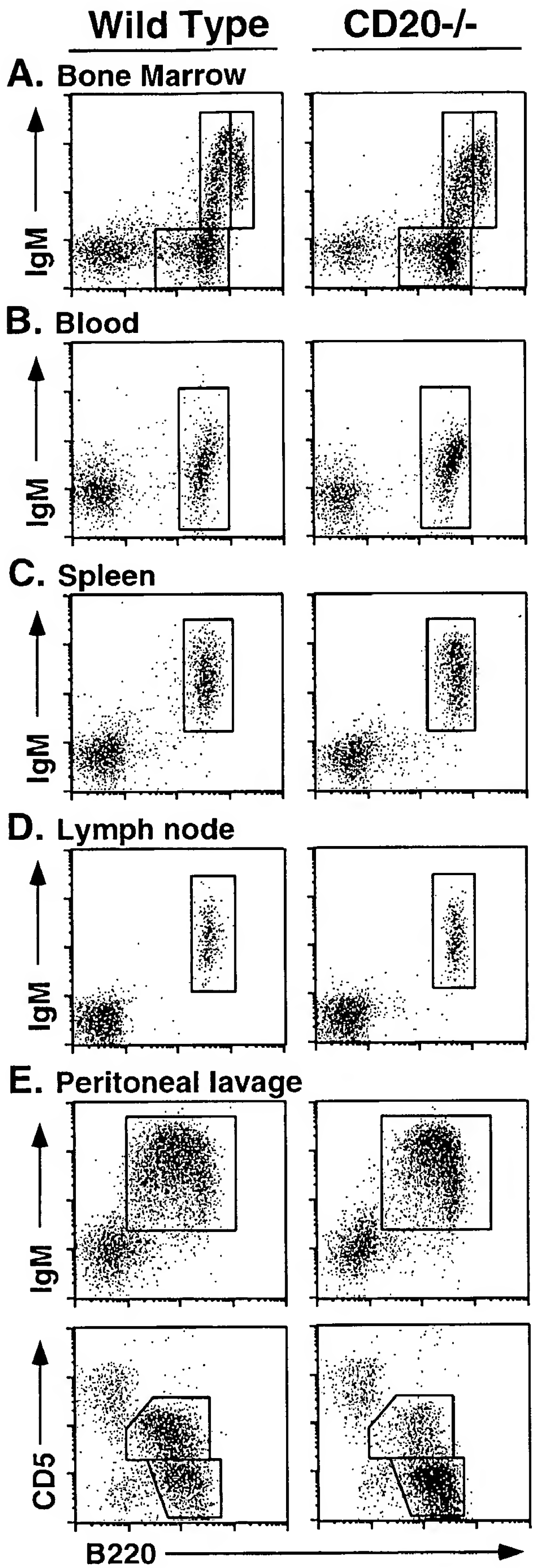
Wild Type
CD20-/-
A. Bone Marrow
B. Blood
C. Spleen
D. Lymph node
E. Peritoneal lavage
IgM
CD5
B220

Figure 11

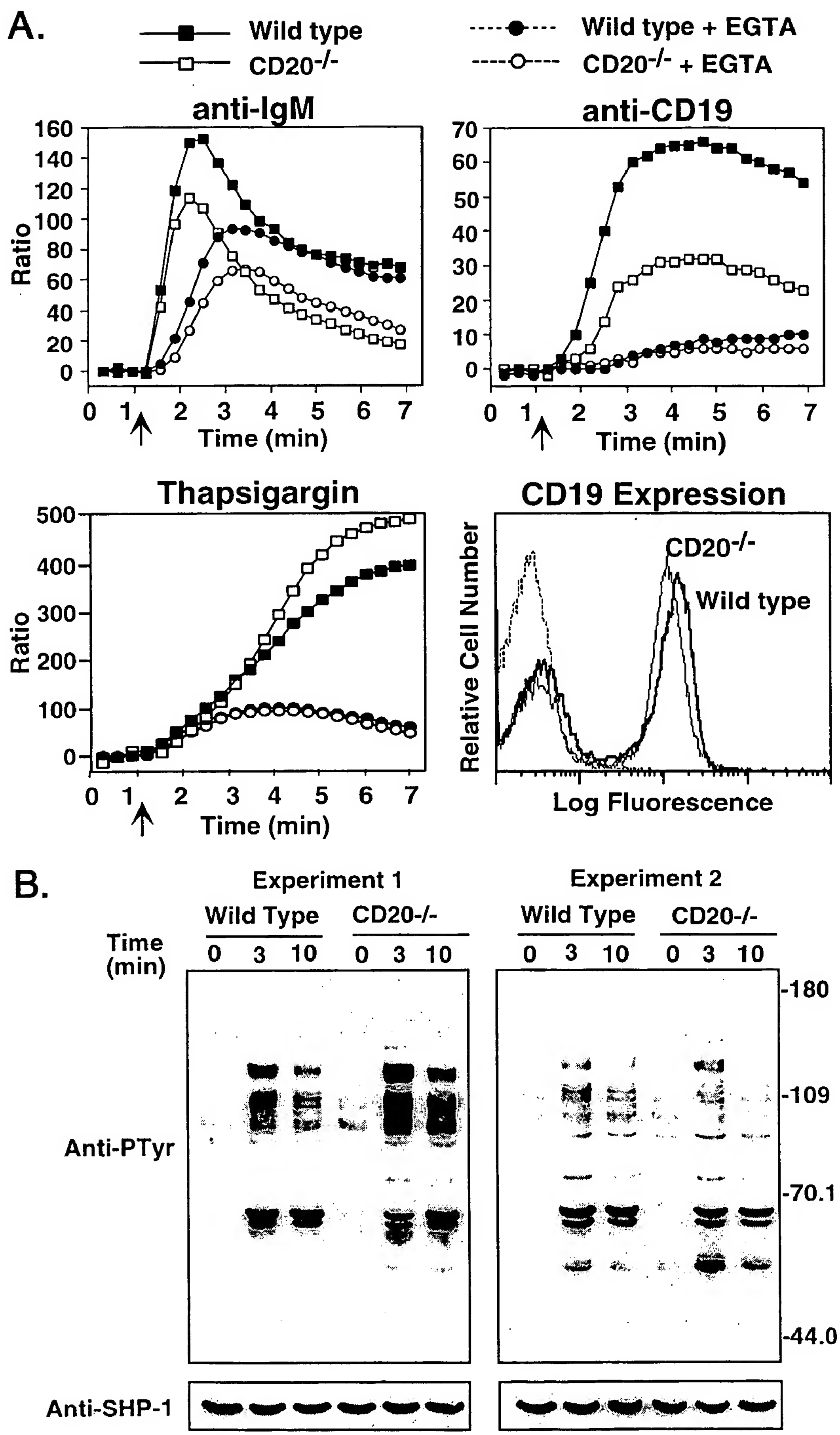
A.
Wild type
CD20-/-
Wild type + EGTA
CD20-/- + EGTA
anti-IgM
Ratio
Time (min)
anti-CD19
Time (min)
Thapsigargin
Ratio
Time (min)
CD19 Expression
CD20-/-
Wild type
Relative Cell Number
Log Fluorescence
B.
Experiment 1
Experiment 2
Wild Type
CD20-/-
Time (min)
0 3 10
Anti-PTyr
Anti-SHP-1
-180
-109
-70.1
-44.0

Figure 12

Sequence Listing

<110> Tedder, Thomas

Liang, Yinghua

<120> Identification of Novel MS4A Gene Family Members Expressed by Hematopoietic Cells

<130> 180/132

<160> 81

<170> PatentIn version 3.0

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<211> 1619

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<213> Homo sapiens

<220>

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ctttggaaca acttaaataa gtcaaatata cttggagctt taaaaattaa aaggagagag     120

attcgagcac cttttctgct gcc atg aca acc atg caa gga atg gaa cag gcc     173
                          Met Thr Thr Met Gln Gly Met Glu Gln Ala
                          1               5                   10
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atg cca ggg gct ggc cct ggt gtg ccc cag ctg gga aac atg gct gtc       221
Met Pro Gly Ala Gly Pro Gly Val Pro Gln Leu Gly Asn Met Ala Val
                15                  20                  25

ata cat tca cat ctg tgg aaa gga ttg caa gag aag ttc ttg aag gga       269
Ile His Ser His Leu Trp Lys Gly Leu Gln Glu Lys Phe Leu Lys Gly
            30                  35                  40

gaa ccc aaa gtc ctt ggg gtt gtg cag att ctg act gcc ctg atg agc       317
Glu Pro Lys Val Leu Gly Val Val Gln Ile Leu Thr Ala Leu Met Ser
        45                  50                  55

ctt agc atg gga ata aca atg atg tgt atg gca tct aat act tat gga       365
Leu Ser Met Gly Ile Thr Met Met Cys Met Ala Ser Asn Thr Tyr Gly
    60                  65                  70

agt aac cct att tcc gtg tat atc ggg tac aca att tgg ggg tca gta       413
Ser Asn Pro Ile Ser Val Tyr Ile Gly Tyr Thr Ile Trp Gly Ser Val
75                  80                  85                  90

atg ttt att att tca gga tcc ttg tca att gca gca gga att aga act       461
Met Phe Ile Ile Ser Gly Ser Leu Ser Ile Ala Ala Gly Ile Arg Thr
                95                  100                 105

aca aaa ggc ctg gtc cga ggt agt cta gga atg aat atc acc agc tct       509
Thr Lys Gly Leu Val Arg Gly Ser Leu Gly Met Asn Ile Thr Ser Ser
            110                 115                 120

gta ctg gct gca tca ggg atc tta atc aac aca ttt agc ttg gcg ttt       557
Val Leu Ala Ala Ser Gly Ile Leu Ile Asn Thr Phe Ser Leu Ala Phe
        125                 130                 135

tat tca ttc cat cac cct tac tgt aac tac tat ggc aac tca aat aat       605
Tyr Ser Phe His His Pro Tyr Cys Asn Tyr Tyr Gly Asn Ser Asn Asn
    140                 145                 150

tgt cat ggg act atg tcc atc tta atg ggt ctg gat ggc atg gtg ctc       653
Cys His Gly Thr Met Ser Ile Leu Met Gly Leu Asp Gly Met Val Leu
155                 160                 165                 170

ctc tta agt gtg ctg gaa ttc tgc att gct gtg tcc ctc tct gcc ttt       701
Leu Leu Ser Val Leu Glu Phe Cys Ile Ala Val Ser Leu Ser Ala Phe
                175                 180                 185

gga tgt aaa gtg ctc tgt tgt acc cct ggt ggg gtt gtg tta att ctg       749
Gly Cys Lys Val Leu Cys Cys Thr Pro Gly Gly Val Val Leu Ile Leu
            190                 195                 200

cca tca cat tct cac atg gca gaa aca gca tct ccc aca cca ctt aat       797
Pro Ser His Ser His Met Ala Glu Thr Ala Ser Pro Thr Pro Leu Asn
        205                 210                 215

gag gtt tga ggccaccaaa agatcaacag acaaatgctc cagaaatcta               846
Glu Val
    220

tgctgactgt gacacaagag cctcacatga gaaattacca gtatccaact tcgatactga     906

tagacttgtt gatattatta ttatatgtaa tccaattatg aactgtgtgt gtatagagag     966
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ataataaatt caaaattatg ttctcatttt tttccctgga actcaataac tcatttcact 1026

ggctctttat cgagagtact agaagttaaa ttaataaata atgcatttaa tgaggcaaca 1086

gcacttgaaa gtttttcatt catcataaga actttatata aaggcattac attggcaaat 1146

aaggtttgga agcagaagag caaaaaaaag atattgttaa aatgaggcct ccatgcaaaa 1206

cacatacttc cctcccattt atttaacttt ttttttctcc tacctatggg gaccaaagtg 1266

ctttttcctt caggaagtgg agatgcatgg ccatctcccc ctcccttttt ccttctcctg 1326

cttttctttc cccatagaaa gtaccttgaa gtagcacagt ccgtccttgc atgtgcacga 1386

gctatcattt gagtaaaagt atacatggag taaaaatcat attaagcatc agattcaact 1446

tatattttct atttcatctt cttcctttcc cttctcccac cttctactgg gcataattat 1506

atcttaatca tatatggaaa tgtgcaacat atggtatttg ttaaatacgt ttgtttttat 1566

tgcagagcaa aaataaatca aattagaagc aataaaaaaa aaaaaaaaaa aaa 1619

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<213> Homo sapiens

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Gly Val Pro Gln Leu Gly Asn Met Ala Val Ile His Ser His Leu Trp
20 25 30

Lys Gly Leu Gln Glu Lys Phe Leu Lys Gly Glu Pro Lys Val Leu Gly
35 40 45

Val Val Gln Ile Leu Thr Ala Leu Met Ser Leu Ser Met Gly Ile Thr
50 55 60

Met Met Cys Met Ala Ser Asn Thr Tyr Gly Ser Asn Pro Ile Ser Val
65 70 75 80

Tyr Ile Gly Tyr Thr Ile Trp Gly Ser Val Met Phe Ile Ile Ser Gly
85 90 95

Ser Leu Ser Ile Ala Ala Gly Ile Arg Thr Thr Lys Gly Leu Val Arg
100 105 110

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Gly Ser Leu Gly Met Asn Ile Thr Ser Ser Val Leu Ala Ala Ser Gly
        115                 120                 125

Ile Leu Ile Asn Thr Phe Ser Leu Ala Phe Tyr Ser Phe His His Pro
    130                 135                 140

Tyr Cys Asn Tyr Tyr Gly Asn Ser Asn Asn Cys His Gly Thr Met Ser
145                 150                 155                 160

Ile Leu Met Gly Leu Asp Gly Met Val Leu Leu Leu Ser Val Leu Glu
                165                 170                 175

Phe Cys Ile Ala Val Ser Leu Ser Ala Phe Gly Cys Lys Val Leu Cys
            180                 185                 190

Cys Thr Pro Gly Gly Val Val Leu Ile Leu Pro Ser His Ser His Met
        195                 200                 205

Ala Glu Thr Ala Ser Pro Thr Pro Leu Asn Glu Val
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<213>  Homo sapiens


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1               5                   10                  15

gat gtg ccc cag ctg gga aac ata gat gtc ata cat tca tat ctg tgt       96
Asp Val Pro Gln Leu Gly Asn Ile Asp Val Ile His Ser Tyr Leu Cys
            20                  25                  30

aaa gga ttg caa gag aag ttc ttc aag agg aaa ccc aaa gtc ctt ggg      144
Lys Gly Leu Gln Glu Lys Phe Phe Lys Arg Lys Pro Lys Val Leu Gly
        35                  40                  45
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gtt gtg cgg att ctg att gcc ttg atg agc ctt agc atg gga ata ata      192
Val Val Arg Ile Leu Ile Ala Leu Met Ser Leu Ser Met Gly Ile Ile
    50                  55                  60

atg atg tgt gtt gca ttt agt tct tat gaa gaa cat ccc att ttt gtg      240
Met Met Cys Val Ala Phe Ser Ser Tyr Glu Glu His Pro Ile Phe Val
65                  70                  75                  80

tat gtt gcg tac aca att tgg ggg tca gtg atg ttt att att tca gta      288
Tyr Val Ala Tyr Thr Ile Trp Gly Ser Val Met Phe Ile Ile Ser Val
                85                  90                  95

tcc tta tca gtt gca gca gga att aga aca aca aaa ggt ctg gtt gga      336
Ser Leu Ser Val Ala Ala Gly Ile Arg Thr Thr Lys Gly Leu Val Gly
            100                 105                 110

ggt agt cta gga aag aat atc acc agt tca gtc ttg gct ata tca ggg      384
Gly Ser Leu Gly Lys Asn Ile Thr Ser Ser Val Leu Ala Ile Ser Gly
        115                 120                 125

atc tta atc aat gca ata agc ttg acg ttt tat tca ttc cgt tac cat      432
Ile Leu Ile Asn Ala Ile Ser Leu Thr Phe Tyr Ser Phe Arg Tyr His
    130                 135                 140

tac tgt aac cac gat cag ttg tca agt aat tgt tac atg act atg tcc      480
Tyr Cys Asn His Asp Gln Leu Ser Ser Asn Cys Tyr Met Thr Met Ser
145                 150                 155                 160

att tta atg ggt acg gat ggc atg gtg ctc ctc tta agt gtg ctg gaa      528
Ile Leu Met Gly Thr Asp Gly Met Val Leu Leu Leu Ser Val Leu Glu
                165                 170                 175

ttc tgc att gct gtg tcc ctc tct gcc ttt gga tgt aaa gtg ctt tgt      576
Phe Cys Ile Ala Val Ser Leu Ser Ala Phe Gly Cys Lys Val Leu Cys
            180                 185                 190

tgt agc ccc agt gag ttt gtg cta att aca cca tca aat tct cac atg      624
Cys Ser Pro Ser Glu Phe Val Leu Ile Thr Pro Ser Asn Ser His Met
        195                 200                 205

gca gaa ata gca tct ccc aca cca ctt aag aca gtt tga                  663
Ala Glu Ile Ala Ser Pro Thr Pro Leu Lys Thr Val
    210                 215                 220
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<211> 220

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<213> Homo sapiens

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Lys Gly Leu Gln Glu Lys Phe Phe Lys Arg Lys Pro Lys Val Leu Gly
        35                  40                  45

Val Val Arg Ile Leu Ile Ala Leu Met Ser Leu Ser Met Gly Ile Ile
    50                  55                  60

Met Met Cys Val Ala Phe Ser Ser Tyr Glu Glu His Pro Ile Phe Val
65                  70                  75                  80

Tyr Val Ala Tyr Thr Ile Trp Gly Ser Val Met Phe Ile Ile Ser Val
                85                  90                  95

Ser Leu Ser Val Ala Ala Gly Ile Arg Thr Thr Lys Gly Leu Val Gly
            100                 105                 110

Gly Ser Leu Gly Lys Asn Ile Thr Ser Ser Val Leu Ala Ile Ser Gly
        115                 120                 125

Ile Leu Ile Asn Ala Ile Ser Leu Thr Phe Tyr Ser Phe Arg Tyr His
    130                 135                 140

Tyr Cys Asn His Asp Gln Leu Ser Ser Asn Cys Tyr Met Thr Met Ser
145                 150                 155                 160

Ile Leu Met Gly Thr Asp Gly Met Val Leu Leu Leu Ser Val Leu Glu
                165                 170                 175

Phe Cys Ile Ala Val Ser Leu Ser Ala Phe Gly Cys Lys Val Leu Cys
            180                 185                 190

Cys Ser Pro Ser Glu Phe Val Leu Ile Thr Pro Ser Asn Ser His Met
        195                 200                 205

Ala Glu Ile Ala Ser Pro Thr Pro Leu Lys Thr Val
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<213> Homo sapiens

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                                                            Met
                                                            1

gat tca agc acc gca cac agt ccg gtg ttt ctg gta ttt cct cca gaa      105
Asp Ser Ser Thr Ala His Ser Pro Val Phe Leu Val Phe Pro Pro Glu
            5                   10                  15

atc act gct tca gaa tat gag tcc aca gaa ctt tca gcc acg acc ttt      153
Ile Thr Ala Ser Glu Tyr Glu Ser Thr Glu Leu Ser Ala Thr Thr Phe
        20                  25                  30

tca act caa agc ccc ttg caa aaa tta ttt gct aga aaa atg aaa atc      201
Ser Thr Gln Ser Pro Leu Gln Lys Leu Phe Ala Arg Lys Met Lys Ile
    35                  40                  45

tta ggg act atc cag atc ctg ttt gga att atg acc ttt tct ttt gga      249
Leu Gly Thr Ile Gln Ile Leu Phe Gly Ile Met Thr Phe Ser Phe Gly
50                  55                  60                  65

gtt atc ttc ctt ttc act ttg tta aaa cca tat cca agg ttt ccc ttt      297
Val Ile Phe Leu Phe Thr Leu Leu Lys Pro Tyr Pro Arg Phe Pro Phe
                70                  75                  80

ata ttt ctt tca gga tat cca ttc tgg ggc tct gtt ttg ttc att aat      345
Ile Phe Leu Ser Gly Tyr Pro Phe Trp Gly Ser Val Leu Phe Ile Asn
            85                  90                  95

tct gga gcc ttc cta att gca gtg aaa aga aaa acc aca gaa act ctg      393
Ser Gly Ala Phe Leu Ile Ala Val Lys Arg Lys Thr Thr Glu Thr Leu
        100                 105                 110

ata ata ttg agc cga ata atg aat ttt ctt agt gcc ctg gga gca ata      441
Ile Ile Leu Ser Arg Ile Met Asn Phe Leu Ser Ala Leu Gly Ala Ile
    115                 120                 125

gct gga atc att ctc ctc aca ttt ggt ttc atc cta gat caa aac tac      489
Ala Gly Ile Ile Leu Leu Thr Phe Gly Phe Ile Leu Asp Gln Asn Tyr
130                 135                 140                 145

att tgt ggt tat tct cac caa aat agt cag tgt aag gct gtt act gtc      537
Ile Cys Gly Tyr Ser His Gln Asn Ser Gln Cys Lys Ala Val Thr Val
```

```
                150                 155                 160

ctg ttc ttg gga att ttg att aca ttg atg act ttc agc att att gaa      585
Leu Phe Leu Gly Ile Leu Ile Thr Leu Met Thr Phe Ser Ile Ile Glu
            165                 170                 175

tta ttc att tct ctg cct ttc tca att ttg ggg tgc cac tca gag gat      633
Leu Phe Ile Ser Leu Pro Phe Ser Ile Leu Gly Cys His Ser Glu Asp
        180                 185                 190

tgt gat tgt gaa caa tgt tgt tga ctagcactgt gagaataaag atgtgttaaa     687
Cys Asp Cys Glu Gln Cys Cys
    195                 200

ataaaaa                                                              694


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<212>  PRT

<213>  Homo sapiens


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            20                  25                  30


Phe Ser Thr Gln Ser Pro Leu Gln Lys Leu Phe Ala Arg Lys Met Lys
        35                  40                  45


Ile Leu Gly Thr Ile Gln Ile Leu Phe Gly Ile Met Thr Phe Ser Phe
    50                  55                  60


Gly Val Ile Phe Leu Phe Thr Leu Leu Lys Pro Tyr Pro Arg Phe Pro
65                  70                  75                  80


Phe Ile Phe Leu Ser Gly Tyr Pro Phe Trp Gly Ser Val Leu Phe Ile
                85                  90                  95


Asn Ser Gly Ala Phe Leu Ile Ala Val Lys Arg Lys Thr Thr Glu Thr
            100                 105                 110


Leu Ile Ile Leu Ser Arg Ile Met Asn Phe Leu Ser Ala Leu Gly Ala
        115                 120                 125
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Ile Ala Gly Ile Ile Leu Leu Thr Phe Gly Phe Ile Leu Asp Gln Asn
130 135 140

Tyr Ile Cys Gly Tyr Ser His Gln Asn Ser Gln Cys Lys Ala Val Thr
145 150 155 160

Val Leu Phe Leu Gly Ile Leu Ile Thr Leu Met Thr Phe Ser Ile Ile
165 170 175

Glu Leu Phe Ile Ser Leu Pro Phe Ser Ile Leu Gly Cys His Ser Glu
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ctttcagaag atgaaggtaa gtagaaaccg ttgatgggac tgagaaacca gagttaaaac 180

ctctttggag cttctgagga ctcagctgga accaacgggc acagttggca acaccatc 238

atg aca tca caa cct gtt ccc aat gag acc atc ata gtg ctc cca tca 286
Met Thr Ser Gln Pro Val Pro Asn Glu Thr Ile Ile Val Leu Pro Ser
1 5 10 15

aat gtc atc aac ttc tcc caa gca gag aaa ccc gaa ccc acc aac cag 334

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Asn Val Ile Asn Phe Ser Gln Ala Glu Lys Pro Glu Pro Thr Asn Gln
            20                  25                  30

ggg cag gat agc ctg aag aaa cat cta cac gca gaa att aaa gtt att      382
Gly Gln Asp Ser Leu Lys Lys His Leu His Ala Glu Ile Lys Val Ile
        35                  40                  45

ggg act atc cag atc ttg tgt ggc atg atg gta ttg agc ttg ggg atc      430
Gly Thr Ile Gln Ile Leu Cys Gly Met Met Val Leu Ser Leu Gly Ile
    50                  55                  60

att ttg gca tct gct tcc ttc tct cca aat ttt acc caa gtg act tct      478
Ile Leu Ala Ser Ala Ser Phe Ser Pro Asn Phe Thr Gln Val Thr Ser
65                  70                  75                  80

aca ctg ttg aac tct gct tac cca ttc ata gga ccc ttt ttt ttt atc      526
Thr Leu Leu Asn Ser Ala Tyr Pro Phe Ile Gly Pro Phe Phe Phe Ile
                85                  90                  95

atc tct ggc tct cta tca atc gcc aca gag aaa agg tta acc aag ctt      574
Ile Ser Gly Ser Leu Ser Ile Ala Thr Glu Lys Arg Leu Thr Lys Leu
            100                 105                 110

ttg gtg cat agc agc ctg gtt gga agc att ctg agt gct ctg tct gcc      622
Leu Val His Ser Ser Leu Val Gly Ser Ile Leu Ser Ala Leu Ser Ala
        115                 120                 125

ctg gtg ggt ttc att atc ctg tct gtc aaa cag gcc acc tta aat cct      670
Leu Val Gly Phe Ile Ile Leu Ser Val Lys Gln Ala Thr Leu Asn Pro
    130                 135                 140

gcc tca ctg cag tgt gag ttg gac aaa aat aat ata cca aca aga agt      718
Ala Ser Leu Gln Cys Glu Leu Asp Lys Asn Asn Ile Pro Thr Arg Ser
145                 150                 155                 160

tat gtt tct tac ttt tat cat gat tca ctt tat acc acg gac tgc tat      766
Tyr Val Ser Tyr Phe Tyr His Asp Ser Leu Tyr Thr Thr Asp Cys Tyr
                165                 170                 175

aca gcc aaa gcc agt ctg gct gga act ctc tct ctg atg ctg att tgc      814
Thr Ala Lys Ala Ser Leu Ala Gly Thr Leu Ser Leu Met Leu Ile Cys
            180                 185                 190

act ctg ctg gaa ttc tgc cta gct gtg ctc act gct gtg ctg cgg tgg      862
Thr Leu Leu Glu Phe Cys Leu Ala Val Leu Thr Ala Val Leu Arg Trp
        195                 200                 205

aaa cag gct tac tct gac ttc cct ggg agt gta ctt ttc ctg cct cac      910
Lys Gln Ala Tyr Ser Asp Phe Pro Gly Ser Val Leu Phe Leu Pro His
    210                 215                 220

agt tac att ggt aat tct ggc atg tcc tca aaa atg act cat gac tgt      958
Ser Tyr Ile Gly Asn Ser Gly Met Ser Ser Lys Met Thr His Asp Cys
225                 230                 235                 240

gga tat gaa gaa cta ttg act tct taa gaaaaaggg agaaatatta            1005
Gly Tyr Glu Glu Leu Leu Thr Ser
                245

atcagaaagt tgattcttat gataatatgg aaaagttaac cattatagaa aagcaaagct   1065
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tgagtttcct aaatgtaagc ttttaaagta atgaacatta aaaaaaacca ttatttcact 1125

gtcaaaaaaa aaaaaaaaaa aaaaaaa 1152

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<211> 248

<212> PRT

<213> Homo sapiens

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Asn Val Ile Asn Phe Ser Gln Ala Glu Lys Pro Glu Pro Thr Asn Gln
20 25 30

Gly Gln Asp Ser Leu Lys Lys His Leu His Ala Glu Ile Lys Val Ile
35 40 45

Gly Thr Ile Gln Ile Leu Cys Gly Met Met Val Leu Ser Leu Gly Ile
50 55 60

Ile Leu Ala Ser Ala Ser Phe Ser Pro Asn Phe Thr Gln Val Thr Ser
65 70 75 80

Thr Leu Leu Asn Ser Ala Tyr Pro Phe Ile Gly Pro Phe Phe Phe Ile
85 90 95

Ile Ser Gly Ser Leu Ser Ile Ala Thr Glu Lys Arg Leu Thr Lys Leu
100 105 110

Leu Val His Ser Ser Leu Val Gly Ser Ile Leu Ser Ala Leu Ser Ala
115 120 125

Leu Val Gly Phe Ile Ile Leu Ser Val Lys Gln Ala Thr Leu Asn Pro
130 135 140

Ala Ser Leu Gln Cys Glu Leu Asp Lys Asn Asn Ile Pro Thr Arg Ser
145 150 155 160

Tyr Val Ser Tyr Phe Tyr His Asp Ser Leu Tyr Thr Thr Asp Cys Tyr
165 170 175

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Thr Ala Lys Ala Ser Leu Ala Gly Thr Leu Ser Leu Met Leu Ile Cys
            180                     185                     190

Thr Leu Leu Glu Phe Cys Leu Ala Val Leu Thr Ala Val Leu Arg Trp
        195                     200                     205

Lys Gln Ala Tyr Ser Asp Phe Pro Gly Ser Val Leu Phe Leu Pro His
    210                     215                     220

Ser Tyr Ile Gly Asn Ser Gly Met Ser Ser Lys Met Thr His Asp Cys
225                     230                     235                     240

Gly Tyr Glu Glu Leu Leu Thr Ser
                245


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<213>  Homo sapiens


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<222>  (17)..(763)


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                  Met Thr Ser Gln Pro Val Pro Asn Glu Thr Ile Ile
                  1               5                   10

gtg ctc cca tca aat gtc atc aac ttc tcc caa gca gag aaa ccc gaa       100
Val Leu Pro Ser Asn Val Ile Asn Phe Ser Gln Ala Glu Lys Pro Glu
        15                  20                  25

ccc acc aac cag ggg cag gat agc ctg aag aaa cat cta cac gca gaa       148
Pro Thr Asn Gln Gly Gln Asp Ser Leu Lys Lys His Leu His Ala Glu
    30                  35                  40

atc aaa gtt att ggg act atc cag atc ttg tgt ggc atg atg gta ttg       196
Ile Lys Val Ile Gly Thr Ile Gln Ile Leu Cys Gly Met Met Val Leu
```

45 50 55 60

agc ttg ggg atc att ttg gca tct gct tcc ttc tct cca aat ttt acc 244
Ser Leu Gly Ile Ile Leu Ala Ser Ala Ser Phe Ser Pro Asn Phe Thr
65 70 75

caa gtg act tct aca ctg ttg aac tct gct tac cca ttc ata gga ccc 292
Gln Val Thr Ser Thr Leu Leu Asn Ser Ala Tyr Pro Phe Ile Gly Pro
80 85 90

ttt ttt ttt atc atc tct ggc tct cta tca atc gcc aca gag aaa agg 340
Phe Phe Phe Ile Ile Ser Gly Ser Leu Ser Ile Ala Thr Glu Lys Arg
95 100 105

tta acc aag ctt ttg gtg cat agc agc ctg gtt gga agc att ctg agt 388
Leu Thr Lys Leu Leu Val His Ser Ser Leu Val Gly Ser Ile Leu Ser
110 115 120

gct ctg tct gcc ctg gtg ggt ttc att atc ctg tct gtc aaa cag gcc 436
Ala Leu Ser Ala Leu Val Gly Phe Ile Ile Leu Ser Val Lys Gln Ala
125 130 135 140

acc tta aat cct gcc tca ctg cag tgt gag ttg gac aaa aat aat ata 484
Thr Leu Asn Pro Ala Ser Leu Gln Cys Glu Leu Asp Lys Asn Asn Ile
145 150 155

cca aca aga agt tat gtt tct tac ttt tat cat gat tca ctt tat acc 532
Pro Thr Arg Ser Tyr Val Ser Tyr Phe Tyr His Asp Ser Leu Tyr Thr
160 165 170

acg gac tgc tat aca gcc aaa gcc agt ctg gct gga tct ctc tct ctg 580
Thr Asp Cys Tyr Thr Ala Lys Ala Ser Leu Ala Gly Ser Leu Ser Leu
175 180 185

atg ctg att tgc act ctg ctg gaa ttc tgc cta gct gtg ctc act gct 628
Met Leu Ile Cys Thr Leu Leu Glu Phe Cys Leu Ala Val Leu Thr Ala
190 195 200

gtg ctg cgg tgg aaa cag gct tac tct gac ttc cct ggg agt gta ctt 676
Val Leu Arg Trp Lys Gln Ala Tyr Ser Asp Phe Pro Gly Ser Val Leu
205 210 215 220

ttc ctg cct cac agt tac att ggt aat tct ggc atg tcc tca aaa atg 724
Phe Leu Pro His Ser Tyr Ile Gly Asn Ser Gly Met Ser Ser Lys Met
225 230 235

act cat gac tgt gga tat gaa gaa cta ttg act tct taa gaaaaaaggg 773
Thr His Asp Cys Gly Tyr Glu Glu Leu Leu Thr Ser
240 245

agaaatatta atcagaaagt tgattcttat gataatatgg aaaagttaac cattatagaa 833

aagcaaagct tgagtttcct aaatgtaagc ttttaaagta atgaacatta aaaaaaacca 893

ttatttcact gtcaaaaaaa aaaaaaaaaa aaaaaaa 930

<210> 10

<211> 248

<212> PRT

<213> Homo sapiens

<400> 10

```
Met Thr Ser Gln Pro Val Pro Asn Glu Thr Ile Ile Val Leu Pro Ser
1               5                   10                  15

Asn Val Ile Asn Phe Ser Gln Ala Glu Lys Pro Glu Pro Thr Asn Gln
            20                  25                  30

Gly Gln Asp Ser Leu Lys Lys His Leu His Ala Glu Ile Lys Val Ile
        35                  40                  45

Gly Thr Ile Gln Ile Leu Cys Gly Met Met Val Leu Ser Leu Gly Ile
    50                  55                  60

Ile Leu Ala Ser Ala Ser Phe Ser Pro Asn Phe Thr Gln Val Thr Ser
65                  70                  75                  80

Thr Leu Leu Asn Ser Ala Tyr Pro Phe Ile Gly Pro Phe Phe Phe Ile
                85                  90                  95

Ile Ser Gly Ser Leu Ser Ile Ala Thr Glu Lys Arg Leu Thr Lys Leu
            100                 105                 110

Leu Val His Ser Ser Leu Val Gly Ser Ile Leu Ser Ala Leu Ser Ala
        115                 120                 125

Leu Val Gly Phe Ile Ile Leu Ser Val Lys Gln Ala Thr Leu Asn Pro
    130                 135                 140

Ala Ser Leu Gln Cys Glu Leu Asp Lys Asn Asn Ile Pro Thr Arg Ser
145                 150                 155                 160

Tyr Val Ser Tyr Phe Tyr His Asp Ser Leu Tyr Thr Thr Asp Cys Tyr
                165                 170                 175

Thr Ala Lys Ala Ser Leu Ala Gly Ser Leu Ser Leu Met Leu Ile Cys
            180                 185                 190

Thr Leu Leu Glu Phe Cys Leu Ala Val Leu Thr Ala Val Leu Arg Trp
        195                 200                 205
```

```
Lys Gln Ala Tyr Ser Asp Phe Pro Gly Ser Val Leu Phe Leu Pro His
    210                 215                 220

Ser Tyr Ile Gly Asn Ser Gly Met Ser Ser Lys Met Thr His Asp Cys
225                 230                 235                 240

Gly Tyr Glu Glu Leu Leu Thr Ser
                245
```

<210> 11

<211> 661

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (15)..(458)

<400> 11

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ttggcaacac catt atg aca tca caa cct att tcc aat gag acc atc ata       50
                Met Thr Ser Gln Pro Ile Ser Asn Glu Thr Ile Ile
                1               5                   10

atg ctc cca tca aat gtc atc aac ttc tcc caa gca gag aaa ccc gaa       98
Met Leu Pro Ser Asn Val Ile Asn Phe Ser Gln Ala Glu Lys Pro Glu
        15                  20                  25

ccc acc aac cag ggg cag gat agc ctg aag aaa cgt cta cag gca aaa      146
Pro Thr Asn Gln Gly Gln Asp Ser Leu Lys Lys Arg Leu Gln Ala Lys
    30                  35                  40

gtc aaa gtt att ggg gtg cat agc agc ctg gct gga agc att ctg agt      194
Val Lys Val Ile Gly Val His Ser Ser Leu Ala Gly Ser Ile Leu Ser
45                  50                  55                  60

gct ctg tct gcc ctg gtg ggt ttc att ctc ctg tct gtc aac ccg gct      242
Ala Leu Ser Ala Leu Val Gly Phe Ile Leu Leu Ser Val Asn Pro Ala
                65                  70                  75

gca tta aat cct gcc tca ttg cag tgt aag ttg gac gaa aag gat ata      290
Ala Leu Asn Pro Ala Ser Leu Gln Cys Lys Leu Asp Glu Lys Asp Ile
            80                  85                  90

cca acc aga ctt ctt ctt tct tat gat tat cat tca cct tac acc atg      338
Pro Thr Arg Leu Leu Leu Ser Tyr Asp Tyr His Ser Pro Tyr Thr Met
        95                  100                 105
```

```
gac tgc cat aga gcc aaa gcc agt ctg gct gga act ctg tct ctg atg      386
Asp Cys His Arg Ala Lys Ala Ser Leu Ala Gly Thr Leu Ser Leu Met
    110                 115                 120

ctg gtt tct act gtg ttg gag ttc tgc cta gct gtg ctc act gct gtg      434
Leu Val Ser Thr Val Leu Glu Phe Cys Leu Ala Val Leu Thr Ala Val
125                 130                 135                 140

ctg cag tgg aaa cag act gtc tga cttccctggt tactcatgtc ctcaaagacg    488
Leu Gln Trp Lys Gln Thr Val
                145

actcatgatg ctggatatga agaactattg acttcttggg aaaaaacgga gaaatattaa   548

ttggaaagtt gatttttatg ataatatgga aaacctaacc attataaaaa agcaaacttg   608

agtttcctaa atgtaagcat ttaaagtaaa tgcatatttg ttttaaaaaa tta          661


<210>  12

<211>  147

<212>  PRT

<213>  Homo sapiens


<400>  12

Met Thr Ser Gln Pro Ile Ser Asn Glu Thr Ile Ile Met Leu Pro Ser
1               5                   10                  15

Asn Val Ile Asn Phe Ser Gln Ala Glu Lys Pro Glu Pro Thr Asn Gln
            20                  25                  30

Gly Gln Asp Ser Leu Lys Lys Arg Leu Gln Ala Lys Val Lys Val Ile
        35                  40                  45

Gly Val His Ser Ser Leu Ala Gly Ser Ile Leu Ser Ala Leu Ser Ala
    50                  55                  60

Leu Val Gly Phe Ile Leu Leu Ser Val Asn Pro Ala Ala Leu Asn Pro
65                  70                  75                  80

Ala Ser Leu Gln Cys Lys Leu Asp Glu Lys Asp Ile Pro Thr Arg Leu
                85                  90                  95

Leu Leu Ser Tyr Asp Tyr His Ser Pro Tyr Thr Met Asp Cys His Arg
            100                 105                 110

Ala Lys Ala Ser Leu Ala Gly Thr Leu Ser Leu Met Leu Val Ser Thr
```

```
        115                 120                 125

Val Leu Glu Phe Cys Leu Ala Val Leu Thr Ala Val Leu Gln Trp Lys
    130                 135                 140

Gln Thr Val
145


<210>  13

<211>  1228

<212>  DNA

<213>  Homo sapiens


<220>

<221>  cDNA

<222>  (1)..(1228)


<220>

<221>  CDS

<222>  (118)..(840)


<400>  13
agattgttct ggcaaggaac agccagtggg aggttccagc tgagcgctcc ccagaggtga      60

gctgatcccc agccacagca cacaggacca ggctgcgaga acagcatcat cagcatc        117

atg cta tta caa tcc caa acc atg ggg gtt tct cac agc ttt aca cca      165
Met Leu Leu Gln Ser Gln Thr Met Gly Val Ser His Ser Phe Thr Pro
1               5                   10                  15

aag ggc atc act atc cct caa aga gag aaa cct gga cac atg tac caa      213
Lys Gly Ile Thr Ile Pro Gln Arg Glu Lys Pro Gly His Met Tyr Gln
            20                  25                  30

aac gaa gat tac ctg cag aac ggg ctg cca aca gaa acc acc gtt ctt      261
Asn Glu Asp Tyr Leu Gln Asn Gly Leu Pro Thr Glu Thr Thr Val Leu
        35                  40                  45

ggg act gtc cag atc ctg tgt tgc ctg ttg att tca agt ctg ggg gcc      309
Gly Thr Val Gln Ile Leu Cys Cys Leu Leu Ile Ser Ser Leu Gly Ala
    50                  55                  60

atc ttg gtt ttt gct ccc tac ccc tcc cac ttc aat cca gca att tcc      357
Ile Leu Val Phe Ala Pro Tyr Pro Ser His Phe Asn Pro Ala Ile Ser
65                  70                  75                  80
```

```
acc act ttg atg tct ggg tac cca ttt tta gga gct ctg tgt ttt ggc      405
Thr Thr Leu Met Ser Gly Tyr Pro Phe Leu Gly Ala Leu Cys Phe Gly
                85                  90                  95

att act gga tcc ctc tca att atc tct gga aaa caa tca act aag ccc      453
Ile Thr Gly Ser Leu Ser Ile Ile Ser Gly Lys Gln Ser Thr Lys Pro
            100                 105                 110

ttt gac ctg agc agc ttg acc tca aat gca gtg agt tct gtt act gca      501
Phe Asp Leu Ser Ser Leu Thr Ser Asn Ala Val Ser Ser Val Thr Ala
        115                 120                 125

gga gca ggc ctc ttc ctc ctt gct gac agc atg gta gcc ctg agg act      549
Gly Ala Gly Leu Phe Leu Leu Ala Asp Ser Met Val Ala Leu Arg Thr
    130                 135                 140

gcc tct caa cat tgt ggc tca gaa atg gat tat cta tcc tca ttg cct      597
Ala Ser Gln His Cys Gly Ser Glu Met Asp Tyr Leu Ser Ser Leu Pro
145                 150                 155                 160

tat tcg gag tac tat tat cca ata tat gaa atc aaa gat tgt ctc ctg      645
Tyr Ser Glu Tyr Tyr Tyr Pro Ile Tyr Glu Ile Lys Asp Cys Leu Leu
                165                 170                 175

acc agt gtc agt tta aca ggt gtc cta gtg gtg atg ctc atc ttc act      693
Thr Ser Val Ser Leu Thr Gly Val Leu Val Val Met Leu Ile Phe Thr
            180                 185                 190

gtg ctg gag ctc tta tta gct gca tac agt tct gtc ttt tgg tgg aaa      741
Val Leu Glu Leu Leu Leu Ala Ala Tyr Ser Ser Val Phe Trp Trp Lys
        195                 200                 205

cag ctc tac tcc aac aac cct ggg agt tca ttt tcc tcg acc cag tca      789
Gln Leu Tyr Ser Asn Asn Pro Gly Ser Ser Phe Ser Ser Thr Gln Ser
    210                 215                 220

caa gat cat atc caa cag gtc aaa aag agt tct tca cgg tct tgg ata      837
Gln Asp His Ile Gln Gln Val Lys Lys Ser Ser Ser Arg Ser Trp Ile
225                 230                 235                 240

taa gtaactcttg gcctcagagg aaggaaaagc aactcaacac tcatggtcaa           890

gtgtgattag actttcctga aatctctgcc attttagata ctgtgaaaca aactaaaaaa    950

aaaaaagctt ttgttttgta tttgtttact atgagtcgtt atttaatttc tcttgaaaat   1010

aatttcctca aagcccaagt caataaatgt tatcagccag tcttccaaaa tggtcataaa   1070

ctttataaac tgctttgggt aaactgagca gaaggtgata cacagaaggg aaaatgtgca   1130

ctcatgctag tgtgaatttg gtaagtcgcg tgactctgca ggctgtttct gtattatttt   1190

cacactcata ttgcttaaat attacatatt agggattg                            1228
```

<210> 14

<211> 240

<212> PRT

<213> Homo sapiens

<400> 14

Met Leu Leu Gln Ser Gln Thr Met Gly Val Ser His Ser Phe Thr Pro
1 5 10 15

Lys Gly Ile Thr Ile Pro Gln Arg Glu Lys Pro Gly His Met Tyr Gln
20 25 30

Asn Glu Asp Tyr Leu Gln Asn Gly Leu Pro Thr Glu Thr Thr Val Leu
35 40 45

Gly Thr Val Gln Ile Leu Cys Cys Leu Leu Ile Ser Ser Leu Gly Ala
50 55 60

Ile Leu Val Phe Ala Pro Tyr Pro Ser His Phe Asn Pro Ala Ile Ser
65 70 75 80

Thr Thr Leu Met Ser Gly Tyr Pro Phe Leu Gly Ala Leu Cys Phe Gly
85 90 95

Ile Thr Gly Ser Leu Ser Ile Ile Ser Gly Lys Gln Ser Thr Lys Pro
100 105 110

Phe Asp Leu Ser Ser Leu Thr Ser Asn Ala Val Ser Ser Val Thr Ala
115 120 125

Gly Ala Gly Leu Phe Leu Leu Ala Asp Ser Met Val Ala Leu Arg Thr
130 135 140

Ala Ser Gln His Cys Gly Ser Glu Met Asp Tyr Leu Ser Ser Leu Pro
145 150 155 160

Tyr Ser Glu Tyr Tyr Tyr Pro Ile Tyr Glu Ile Lys Asp Cys Leu Leu
165 170 175

Thr Ser Val Ser Leu Thr Gly Val Leu Val Val Met Leu Ile Phe Thr
180 185 190

Val Leu Glu Leu Leu Leu Ala Ala Tyr Ser Ser Val Phe Trp Trp Lys
195 200 205

```
Gln Leu Tyr Ser Asn Asn Pro Gly Ser Ser Phe Ser Ser Thr Gln Ser
    210                 215                 220

Gln Asp His Ile Gln Gln Val Lys Lys Ser Ser Ser Arg Ser Trp Ile
225                 230                 235                 240
```

<210> 15

<211> 1369

<212> DNA

<213> Homo sapiens

<220>

<221> cDNA

<222> (1)..(1369)

<220>

<221> CDS

<222> (204)..(956)

<400> 15

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aaacaggaaa taaatacgaa tgaaactgag ctctaagcag catgtaacct ggcctgcatc      60

caggaaatag aggacttcgg atccttctaa ccctaccacc caactggccc cagtacattc     120

attctctcag gaaaaaaaac aaggtcccca cagcaaagaa aaggaatagg atcaagagat     180

acgtggctgc tggcagagca agc atg aat tcg atg act tca gca gtt ccg gtg     233
                          Met Asn Ser Met Thr Ser Ala Val Pro Val
                          1               5                   10

gcc aat tct gtg ttg gtg gtg gca ccc cac aat ggt tat cct gtg acc       281
Ala Asn Ser Val Leu Val Val Ala Pro His Asn Gly Tyr Pro Val Thr
                15                  20                  25

cca gga att atg tct cac gtg ccc ctg tat cca aac agc cag ccg caa       329
Pro Gly Ile Met Ser His Val Pro Leu Tyr Pro Asn Ser Gln Pro Gln
            30                  35                  40

gtc cac cta gtt cct ggg aac cca cct agt ttg gtg tcg aat gtg aat       377
Val His Leu Val Pro Gly Asn Pro Pro Ser Leu Val Ser Asn Val Asn
        45                  50                  55

ggg cag cct gtg cag aaa gct ctg aaa gaa ggc aaa acc ttg ggg gcc       425
Gly Gln Pro Val Gln Lys Ala Leu Lys Glu Gly Lys Thr Leu Gly Ala
    60                  65                  70
```

```
atc cag atc atc att ggc ctg gct cac atc ggc ctc ggc tcc atc atg      473
Ile Gln Ile Ile Ile Gly Leu Ala His Ile Gly Leu Gly Ser Ile Met
75                  80                  85                  90

gcg acg gtt ctc gta ggg gaa tac ctg tct att tca ttc tac gga ggc      521
Ala Thr Val Leu Val Gly Glu Tyr Leu Ser Ile Ser Phe Tyr Gly Gly
                95                  100                 105

ttt ccc ttc tgg gga ggc ttg tgg ttt atc att tca gga tct ctc tcc      569
Phe Pro Phe Trp Gly Gly Leu Trp Phe Ile Ile Ser Gly Ser Leu Ser
            110                 115                 120

gtg gca gca gaa aat cag cca tat tct tat tgc ctg ctg tct ggc agt      617
Val Ala Ala Glu Asn Gln Pro Tyr Ser Tyr Cys Leu Leu Ser Gly Ser
        125                 130                 135

ttg ggc ttg aac atc gtc agt gca atc tgc tct gca gtt gga gtc ata      665
Leu Gly Leu Asn Ile Val Ser Ala Ile Cys Ser Ala Val Gly Val Ile
    140                 145                 150

ctc ttc atc aca gat cta agt att ccc cac cca tat gcc tac ccc gac      713
Leu Phe Ile Thr Asp Leu Ser Ile Pro His Pro Tyr Ala Tyr Pro Asp
155                 160                 165                 170

tat tat cct tac gcc tgg ggt gtg aac cct gga atg gcg att tct ggc      761
Tyr Tyr Pro Tyr Ala Trp Gly Val Asn Pro Gly Met Ala Ile Ser Gly
                175                 180                 185

gtg ctg ctg gtc ttc tgc ctc ctg gag ttt ggc atc gca tgc gca tct      809
Val Leu Leu Val Phe Cys Leu Leu Glu Phe Gly Ile Ala Cys Ala Ser
            190                 195                 200

tcc cac ttt ggc tgc cag ttg gtc tgc tgt caa tca agc aat gtg agt      857
Ser His Phe Gly Cys Gln Leu Val Cys Cys Gln Ser Ser Asn Val Ser
        205                 210                 215

gtc atc tat cca aac atc tat gca gca aac cca gtg atc acc cca gaa      905
Val Ile Tyr Pro Asn Ile Tyr Ala Ala Asn Pro Val Ile Thr Pro Glu
    220                 225                 230

ccg gtg acc tca cca cca agt tat tcc agt gag atc caa gca aat aag      953
Pro Val Thr Ser Pro Pro Ser Tyr Ser Ser Glu Ile Gln Ala Asn Lys
235                 240                 245                 250

taa ggctacagat tctggaagca tctttcactg ggaccaaaag aagtcctcct          1006

ccctttctgg gcttccataa cccaggtcgt tcctgttctg acagctgagg aaacgtctct   1066

cccactgttt gtactctcac cttcattctt caattcagtc taggaaacca tgctgtttct   1126

ctatcaagaa gaagacagag attttaaaca gatgttaacc aagagggact ccctagggca   1186

catgcatcag cacatatgtg ggcatccagc ctctggggcc ttggcacaca cacattcgtg   1246

tgctctgctg catgtgagct tgtgggttag aggaacaaat atctagacat tcaatcttca   1306

ctctttcaat tgtgcattca tttaataaat agatactgag cattcaaaaa aaaaaaaaaa   1366

aaa                                                                 1369
```

<210> 16

<211> 250

<212> PRT

<213> Homo sapiens

<400> 16

```
Met Asn Ser Met Thr Ser Ala Val Pro Val Ala Asn Ser Val Leu Val
1               5                   10                  15

Val Ala Pro His Asn Gly Tyr Pro Val Thr Pro Gly Ile Met Ser His
            20                  25                  30

Val Pro Leu Tyr Pro Asn Ser Gln Pro Gln Val His Leu Val Pro Gly
        35                  40                  45

Asn Pro Pro Ser Leu Val Ser Asn Val Asn Gly Gln Pro Val Gln Lys
    50                  55                  60

Ala Leu Lys Glu Gly Lys Thr Leu Gly Ala Ile Gln Ile Ile Ile Gly
65                  70                  75                  80

Leu Ala His Ile Gly Leu Gly Ser Ile Met Ala Thr Val Leu Val Gly
                85                  90                  95

Glu Tyr Leu Ser Ile Ser Phe Tyr Gly Gly Phe Pro Phe Trp Gly Gly
            100                 105                 110

Leu Trp Phe Ile Ile Ser Gly Ser Leu Ser Val Ala Ala Glu Asn Gln
        115                 120                 125

Pro Tyr Ser Tyr Cys Leu Leu Ser Gly Ser Leu Gly Leu Asn Ile Val
    130                 135                 140

Ser Ala Ile Cys Ser Ala Val Gly Val Ile Leu Phe Ile Thr Asp Leu
145                 150                 155                 160

Ser Ile Pro His Pro Tyr Ala Tyr Pro Asp Tyr Tyr Pro Tyr Ala Trp
                165                 170                 175

Gly Val Asn Pro Gly Met Ala Ile Ser Gly Val Leu Leu Val Phe Cys
            180                 185                 190
```

Leu Leu Glu Phe Gly Ile Ala Cys Ala Ser Ser His Phe Gly Cys Gln
195 200 205

Leu Val Cys Cys Gln Ser Ser Asn Val Ser Val Ile Tyr Pro Asn Ile
210 215 220

Tyr Ala Ala Asn Pro Val Ile Thr Pro Glu Pro Val Thr Ser Pro Pro
225 230 235 240

Ser Tyr Ser Ser Glu Ile Gln Ala Asn Lys
245 250

<210> 17

<211> 726

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(726)

<400> 17
atg aaa gca gaa gcc aca gtt att ccc agc cgt tgt gct agg ggg ctc 48
Met Lys Ala Glu Ala Thr Val Ile Pro Ser Arg Cys Ala Arg Gly Leu
1 5 10 15

cca tca tgg caa gtc ctc agc cca gtc cag ccc tgg cag aca agt gca 96
Pro Ser Trp Gln Val Leu Ser Pro Val Gln Pro Trp Gln Thr Ser Ala
20 25 30

ccc cag aac acg acc cag ccc aag ctc ctg gct cca cac cag cac gag 144
Pro Gln Asn Thr Thr Gln Pro Lys Leu Leu Ala Pro His Gln His Glu
35 40 45

aag tcc cag aag aag agc agc ctt ctt aag gag ctg ggg gcc ttc cac 192
Lys Ser Gln Lys Lys Ser Ser Leu Leu Lys Glu Leu Gly Ala Phe His
50 55 60

atc acc atc gct ctg ctg cac ctg gtc ttt ggg ggc tac ctg gcc tct 240
Ile Thr Ile Ala Leu Leu His Leu Val Phe Gly Gly Tyr Leu Ala Ser
65 70 75 80

ata gtc aag aac ctt cac ctg gtg gtg ctg aag tct tgg tat cca ttc 288
Ile Val Lys Asn Leu His Leu Val Val Leu Lys Ser Trp Tyr Pro Phe
85 90 95

```
tgg ggg gct gcc tct ttt ctc att tca ggg atc ttg gcg ata aca atg      336
Trp Gly Ala Ala Ser Phe Leu Ile Ser Gly Ile Leu Ala Ile Thr Met
            100                 105                 110

aag acc ttt tct aaa act tac ctg aag atg ttg tgc ctg atg aca aac      384
Lys Thr Phe Ser Lys Thr Tyr Leu Lys Met Leu Cys Leu Met Thr Asn
        115                 120                 125

ctc atc agc ctc ttt tgc gtg ctg tct ggc ctc ttc gtc atc tcc aag      432
Leu Ile Ser Leu Phe Cys Val Leu Ser Gly Leu Phe Val Ile Ser Lys
    130                 135                 140

gat ctc ttt ctg gag agc cca ttt gag tcc ccg atc tgg aga atg tac      480
Asp Leu Phe Leu Glu Ser Pro Phe Glu Ser Pro Ile Trp Arg Met Tyr
145                 150                 155                 160

ccc aac tcc acg gtc cac atc cag agg ctg gag ctg gcc ttg ctc tgc      528
Pro Asn Ser Thr Val His Ile Gln Arg Leu Glu Leu Ala Leu Leu Cys
                165                 170                 175

ttc act gtc cta gag ctc ttc ctg cca gtg ccc aca gct gtc aca gcc      576
Phe Thr Val Leu Glu Leu Phe Leu Pro Val Pro Thr Ala Val Thr Ala
            180                 185                 190

tgg aga ggg gac tgc cca tct gca aag aat gat gat gca tgc ctt gtt      624
Trp Arg Gly Asp Cys Pro Ser Ala Lys Asn Asp Asp Ala Cys Leu Val
        195                 200                 205

ccg aat aca cca ttg cat ctc aaa ggc ctg ccg gtg gag ccc ccg cca      672
Pro Asn Thr Pro Leu His Leu Lys Gly Leu Pro Val Glu Pro Pro Pro
    210                 215                 220

tcc tac cag agt gtg att caa ggc gac gca caa cac aag caa cat cag      720
Ser Tyr Gln Ser Val Ile Gln Gly Asp Ala Gln His Lys Gln His Gln
225                 230                 235                 240

agg tga                                                              726
Arg
```

<210> 18

<211> 241

<212> PRT

<213> Homo sapiens

<400> 18

```
Met Lys Ala Glu Ala Thr Val Ile Pro Ser Arg Cys Ala Arg Gly Leu
1               5                   10                  15

Pro Ser Trp Gln Val Leu Ser Pro Val Gln Pro Trp Gln Thr Ser Ala
            20                  25                  30

Pro Gln Asn Thr Thr Gln Pro Lys Leu Leu Ala Pro His Gln His Glu
```

35 40 45

Lys Ser Gln Lys Lys Ser Ser Leu Leu Lys Glu Leu Gly Ala Phe His
50 55 60

Ile Thr Ile Ala Leu Leu His Leu Val Phe Gly Gly Tyr Leu Ala Ser
65 70 75 80

Ile Val Lys Asn Leu His Leu Val Val Leu Lys Ser Trp Tyr Pro Phe
85 90 95

Trp Gly Ala Ala Ser Phe Leu Ile Ser Gly Ile Leu Ala Ile Thr Met
100 105 110

Lys Thr Phe Ser Lys Thr Tyr Leu Lys Met Leu Cys Leu Met Thr Asn
115 120 125

Leu Ile Ser Leu Phe Cys Val Leu Ser Gly Leu Phe Val Ile Ser Lys
130 135 140

Asp Leu Phe Leu Glu Ser Pro Phe Glu Ser Pro Ile Trp Arg Met Tyr
145 150 155 160

Pro Asn Ser Thr Val His Ile Gln Arg Leu Glu Leu Ala Leu Leu Cys
165 170 175

Phe Thr Val Leu Glu Leu Phe Leu Pro Val Pro Thr Ala Val Thr Ala
180 185 190

Trp Arg Gly Asp Cys Pro Ser Ala Lys Asn Asp Asp Ala Cys Leu Val
195 200 205

Pro Asn Thr Pro Leu His Leu Lys Gly Leu Pro Val Glu Pro Pro Pro
210 215 220

Ser Tyr Gln Ser Val Ile Gln Gly Asp Ala Gln His Lys Gln His Gln
225 230 235 240

Arg

<210> 19

<211> 518

<212> DNA

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<213>  Mus musculus


<220>

<221>  cDNA

<222>  (1)..(518)


<220>

<221>  CDS

<222>  (148)..(516)


<400>  19
taagcgctga ggaggaagag actgctggtt ttgggggaca gactctggtg gtcattactg      60

tctcctcttc tgtaatgagt tggacttgca ggggaaggac ttgtcaaatc ctgaattctt     120

caaggtggta cctcatcagg cagacac atg aag cca gag gag act ggt ggt tct     174
                              Met Lys Pro Glu Glu Thr Gly Gly Ser
                              1               5

gtt tat cag ccc ttg gat gag tca cgc cat gtt caa cga ggt gta ctg       222
Val Tyr Gln Pro Leu Asp Glu Ser Arg His Val Gln Arg Gly Val Leu
10                  15                  20                  25

caa gcc ctc ggg gcc atc cag atc ctg aat gga ata ctg att cta gct       270
Gln Ala Leu Gly Ala Ile Gln Ile Leu Asn Gly Ile Leu Ile Leu Ala
                30                  35                  40

ctc gga att ttt ctg gtt tgt tta caa cac gtg tcc cac cac ttc agg       318
Leu Gly Ile Phe Leu Val Cys Leu Gln His Val Ser His His Phe Arg
            45                  50                  55

cat ttc ttc ttc ttc acc ttc tac aca ggc tac cca ctg tgg ggt gct       366
His Phe Phe Phe Phe Thr Phe Tyr Thr Gly Tyr Pro Leu Trp Gly Ala
        60                  65                  70

gtg ttt ttt atc agc tca gga tcc ttg act gtt gcc gca ggg aga aac       414
Val Phe Phe Ile Ser Ser Gly Ser Leu Thr Val Ala Ala Gly Arg Asn
    75                  80                  85

ccc aca cga atg ctg atg caa aac agt ttt ggg ata aac att gcc agt       462
Pro Thr Arg Met Leu Met Gln Asn Ser Phe Gly Ile Asn Ile Ala Ser
90                  95                  100                 105


act aca att gca ttt gtt ggg act gtt ttc ctt tct gtg cat ttg gca       510
Thr Thr Ile Ala Phe Val Gly Thr Val Phe Leu Ser Val His Leu Ala
                110                 115                 120

ttc aat ac                                                            518
Phe Asn
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<210> 20

<211> 123

<212> PRT

<213> Mus musculus

<400> 20

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Met Lys Pro Glu Glu Thr Gly Gly Ser Val Tyr Gln Pro Leu Asp Glu
1               5                   10                  15

Ser Arg His Val Gln Arg Gly Val Leu Gln Ala Leu Gly Ala Ile Gln
            20                  25                  30

Ile Leu Asn Gly Ile Leu Ile Leu Ala Leu Gly Ile Phe Leu Val Cys
        35                  40                  45

Leu Gln His Val Ser His His Phe Arg His Phe Phe Phe Phe Thr Phe
    50                  55                  60

Tyr Thr Gly Tyr Pro Leu Trp Gly Ala Val Phe Phe Ile Ser Ser Gly
65                  70                  75                  80

Ser Leu Thr Val Ala Ala Gly Arg Asn Pro Thr Arg Met Leu Met Gln
                85                  90                  95

Asn Ser Phe Gly Ile Asn Ile Ala Ser Thr Thr Ile Ala Phe Val Gly
            100                 105                 110

Thr Val Phe Leu Ser Val His Leu Ala Phe Asn
        115                 120
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<210> 21

<211> 1228

<212> DNA

<213> Mus musculus

<220>

<221> cDNA

<222> (1)..(1228)

<220>

<221> CDS

<222> (98)..(778)

<400> 21
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ttccattgtc tgtactgttt ctgctgctct ggaaacc atg caa gga cag gaa cag      115
                                         Met Gln Gly Gln Glu Gln
                                         1               5

acc acc atg gca gtg gtt cct gga gtt gct gtg cct tca aag aat tct      163
Thr Thr Met Ala Val Val Pro Gly Val Ala Val Pro Ser Lys Asn Ser
            10                  15                  20

gtt atg aca tca caa atg tgg aat gag aag aaa gag aaa ttc ttg aag      211
Val Met Thr Ser Gln Met Trp Asn Glu Lys Lys Glu Lys Phe Leu Lys
        25                  30                  35

ggg gaa ccc aaa gtc ctt ggg gtt tta caa gtg atg att gct atc ata      259
Gly Glu Pro Lys Val Leu Gly Val Leu Gln Val Met Ile Ala Ile Ile
    40                  45                  50

aac ctc agc tta gga ata ata att ttg aca act tta ttt tct gaa cta      307
Asn Leu Ser Leu Gly Ile Ile Ile Leu Thr Thr Leu Phe Ser Glu Leu
55                  60                  65                  70

ccc act tca gtg atg tta atg gtc cca att tgg gga tca ata atg ttc      355
Pro Thr Ser Val Met Leu Met Val Pro Ile Trp Gly Ser Ile Met Phe
                75                  80                  85

att gtc tcc gga tcc ctg tcc att gca gca gga gtg aca cct aca aaa      403
Ile Val Ser Gly Ser Leu Ser Ile Ala Ala Gly Val Thr Pro Thr Lys
            90                  95                  100

tgc ctg atc gtt gcc agt cta act ctg aac act atc acc tct gtg ttg      451
Cys Leu Ile Val Ala Ser Leu Thr Leu Asn Thr Ile Thr Ser Val Leu
        105                 110                 115

gct gca act gca agc ata atg ggt gta gtc agt gtg gct gtg ggt tca      499
Ala Ala Thr Ala Ser Ile Met Gly Val Val Ser Val Ala Val Gly Ser
    120                 125                 130

cag ttt ccg ttt cgg tat aat tat aca atc acc aag ggt ttg gat gtt      547
Gln Phe Pro Phe Arg Tyr Asn Tyr Thr Ile Thr Lys Gly Leu Asp Val
135                 140                 145                 150

ttg atg tta att ttc aat atg cta gaa ttc tgc ctt gct gtg tcc gtc      595
Leu Met Leu Ile Phe Asn Met Leu Glu Phe Cys Leu Ala Val Ser Val
                155                 160                 165

tct gca ttt gga tgt gaa gct tcc tgt tgt aac tcc cgt gag gtt ctt      643
Ser Ala Phe Gly Cys Glu Ala Ser Cys Cys Asn Ser Arg Glu Val Leu
```

```
            170                 175                 180

gta gtg cta cca tca aat cct gtt gag aca gtg atg gca ccc cca atg      691
Val Val Leu Pro Ser Asn Pro Val Glu Thr Val Met Ala Pro Pro Met
        185                 190                 195

aca ctt caa cca ttg cta cca tca gaa cac caa ggg acc aat gtt cca      739
Thr Leu Gln Pro Leu Leu Pro Ser Glu His Gln Gly Thr Asn Val Pro
    200                 205                 210

gga aat gtg tac aag aac cac cca gga gaa ata gtc taa ttttgatgtg       788
Gly Asn Val Tyr Lys Asn His Pro Gly Glu Ile Val
215                 220                 225

tgtgtgtgtg tatttcccta ggatattaac acttcattgc actggctttt gaggtgaata    848

ttagatttac tgtaagtatg taagtcaagc acttattagg tcaacaacac ttcaacatat    908

tatattcatt gtatgtacaa ggggcaatga atttgcaaag atgttttgaa agcaaacaga    968

aaaaaaaaaa aaacaaccaa acaaaagacc tcttagtgaa atgaggtctc tttgcaaaga   1028

ctaaaaaact ggagttcaca tttttggggg tggggggggc ttttgctaaa taagtagatt   1088

tagatgcttt tgatcaagta caaactcata aagtatgtaa gaaattacta atgataggaa   1148

ccaattttaa tctcatatgt aacatagtgt ataatttaat agatctggta aaaatttata   1208

ataaagagaa atgcctgaaa                                               1228


<210>  22

<211>  226

<212>  PRT

<213>  Mus musculus


<400>  22

Met Gln Gly Gln Glu Gln Thr Thr Met Ala Val Val Pro Gly Val Ala
1               5                   10                  15

Val Pro Ser Lys Asn Ser Val Met Thr Ser Gln Met Trp Asn Glu Lys
            20                  25                  30

Lys Glu Lys Phe Leu Lys Gly Glu Pro Lys Val Leu Gly Val Leu Gln
        35                  40                  45

Val Met Ile Ala Ile Ile Asn Leu Ser Leu Gly Ile Ile Ile Leu Thr
    50                  55                  60

Thr Leu Phe Ser Glu Leu Pro Thr Ser Val Met Leu Met Val Pro Ile
65                  70                  75                  80
```

```
Trp Gly Ser Ile Met Phe Ile Val Ser Gly Ser Leu Ser Ile Ala Ala
                85                  90                  95

Gly Val Thr Pro Thr Lys Cys Leu Ile Val Ala Ser Leu Thr Leu Asn
            100                 105                 110

Thr Ile Thr Ser Val Leu Ala Ala Thr Ala Ser Ile Met Gly Val Val
        115                 120                 125

Ser Val Ala Val Gly Ser Gln Phe Pro Phe Arg Tyr Asn Tyr Thr Ile
    130                 135                 140

Thr Lys Gly Leu Asp Val Leu Met Leu Ile Phe Asn Met Leu Glu Phe
145                 150                 155                 160

Cys Leu Ala Val Ser Val Ser Ala Phe Gly Cys Glu Ala Ser Cys Cys
                165                 170                 175

Asn Ser Arg Glu Val Leu Val Val Leu Pro Ser Asn Pro Val Glu Thr
            180                 185                 190

Val Met Ala Pro Pro Met Thr Leu Gln Pro Leu Leu Pro Ser Glu His
        195                 200                 205

Gln Gly Thr Asn Val Pro Gly Asn Val Tyr Lys Asn His Pro Gly Glu
    210                 215                 220

Ile Val
225


<210>  23

<211>  1480

<212>  DNA

<213>  Mus musculus


<220>

<221>  cDNA

<222>  (1)..(1480)
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<220>

<221> CDS

<222> (107)..(787)

<400> 23
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tcagatcctt cccattgtct gtactgtttc tgctgccctg gaaacc atg caa gga 115
Met Gln Gly
1

cag gaa cag acc acc atg gca gtg gtt cct gga ggt gct cca cct tca 163
Gln Glu Gln Thr Thr Met Ala Val Val Pro Gly Gly Ala Pro Pro Ser
5 10 15

gag aat tct gtt atg aaa tca caa atg tgg aac gag aac aag gag aaa 211
Glu Asn Ser Val Met Lys Ser Gln Met Trp Asn Glu Asn Lys Glu Lys
20 25 30 35

ttc ttg aag ggg gaa ccc aaa gtc ctt ggg gtt gta caa gtt atg att 259
Phe Leu Lys Gly Glu Pro Lys Val Leu Gly Val Val Gln Val Met Ile
40 45 50

gct ctc ata aac ctc agc ttc gga ata ata att ttg gca aat cta tct 307
Ala Leu Ile Asn Leu Ser Phe Gly Ile Ile Ile Leu Ala Asn Leu Ser
55 60 65

tct gaa cca ctc att tct gtg gtc tta atg gct cca att tgg gga cca 355
Ser Glu Pro Leu Ile Ser Val Val Leu Met Ala Pro Ile Trp Gly Pro
70 75 80

ata atg ttc att gtc tca gga tcc ctg tca att gca gca gga gtg aga 403
Ile Met Phe Ile Val Ser Gly Ser Leu Ser Ile Ala Ala Gly Val Arg
85 90 95

cct aca aaa aag ctg atc atc agc agt cta act ctg aac act atc acc 451
Pro Thr Lys Lys Leu Ile Ile Ser Ser Leu Thr Leu Asn Thr Ile Thr
100 105 110 115

tct gtg ttg gct gca act gca agc ata atg ggt gta gtc agt gtg gct 499
Ser Val Leu Ala Ala Thr Ala Ser Ile Met Gly Val Val Ser Val Ala
120 125 130

gtg ggt tca cag ttt ccg ttt cgg tat aat tat aca atc acc aag ggt 547
Val Gly Ser Gln Phe Pro Phe Arg Tyr Asn Tyr Thr Ile Thr Lys Gly
135 140 145

ttg gat att ttg atg tta att tta aat atg cta gaa ttc tgc att gct 595
Leu Asp Ile Leu Met Leu Ile Leu Asn Met Leu Glu Phe Cys Ile Ala
150 155 160

gtg tcc atc tct gct ttt gga tgt aaa gct tcc tgt tgt aac tcc agc 643
Val Ser Ile Ser Ala Phe Gly Cys Lys Ala Ser Cys Cys Asn Ser Ser
165 170 175

gag gtt ctt gta gtg cta cca tca aat cct gct gtg act gtg atg gca 691

Glu Val Leu Val Val Leu Pro Ser Asn Pro Ala Val Thr Val Met Ala
180 185 190 195

ccc cct gtg acg ctt caa cca ttg cca cca tca gaa cac caa ggg aaa 739
Pro Pro Val Thr Leu Gln Pro Leu Pro Pro Ser Glu His Gln Gly Lys
200 205 210

aat gtt cca gaa aat gta tat aag aac cac tca gaa gaa ata gtc taa 787
Asn Val Pro Glu Asn Val Tyr Lys Asn His Ser Glu Glu Ile Val
215 220 225

ttctcttgtg tgtatgtgaa tgtgtgtgtg tgtgtgtgtg tgtgtgtgta tgcatttccc 847

tgggaaatta acacttcatt gccctggctt ttggggggaa tatcagacat gccataactt 907

tgtaaatcta acatctacta gtacaacaac actttaacat cctccattaa ttgtatgtac 967

aaggggcaat gaattagcaa agatgttttg aaagcaaaat gaaacaaaca aacaagccag 1027

cacacaaaaa aagacctgtt ttgtcttttg caaagattaa agaaaggcgt tcacatttct 1087

ttggggcttt tgctaaataa gtggatttag ctgcttttga tcatgtacaa acacatgaat 1147

tatgtatatt atattactaa tgataggaac caatttttat ctctgtgcat attcatcata 1207

taataacata ctctatactg taattgatat ggtaaaaatt ataataaaga gaagtgactg 1267

aaatcggaat acacaaacac acacacacac acacatttac atacatagtg tatatctctg 1327

tatatacata gtacgtgtat ggtgtgcatg tagagattat agaaggtata catacacata 1387

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tcccctaagg ctcacatctt tgaacagttg gtc 1480

<210> 24

<211> 226

<212> PRT

<213> Mus musculus

<400> 24

Met Gln Gly Gln Glu Gln Thr Thr Met Ala Val Val Pro Gly Gly Ala
1 5 10 15

Pro Pro Ser Glu Asn Ser Val Met Lys Ser Gln Met Trp Asn Glu Asn
20 25 30

Lys Glu Lys Phe Leu Lys Gly Glu Pro Lys Val Leu Gly Val Val Gln
35 40 45

Val Met Ile Ala Leu Ile Asn Leu Ser Phe Gly Ile Ile Ile Leu Ala

```
    50                  55                  60

Asn Leu Ser Ser Glu Pro Leu Ile Ser Val Val Leu Met Ala Pro Ile
65                  70                  75                  80

Trp Gly Pro Ile Met Phe Ile Val Ser Gly Ser Leu Ser Ile Ala Ala
                85                  90                  95

Gly Val Arg Pro Thr Lys Lys Leu Ile Ile Ser Ser Leu Thr Leu Asn
            100                 105                 110

Thr Ile Thr Ser Val Leu Ala Ala Thr Ala Ser Ile Met Gly Val Val
        115                 120                 125

Ser Val Ala Val Gly Ser Gln Phe Pro Phe Arg Tyr Asn Tyr Thr Ile
    130                 135                 140

Thr Lys Gly Leu Asp Ile Leu Met Leu Ile Leu Asn Met Leu Glu Phe
145                 150                 155                 160

Cys Ile Ala Val Ser Ile Ser Ala Phe Gly Cys Lys Ala Ser Cys Cys
                165                 170                 175

Asn Ser Ser Glu Val Leu Val Val Leu Pro Ser Asn Pro Ala Val Thr
            180                 185                 190

Val Met Ala Pro Pro Val Thr Leu Gln Pro Leu Pro Pro Ser Glu His
        195                 200                 205

Gln Gly Lys Asn Val Pro Glu Asn Val Tyr Lys Asn His Ser Glu Glu
    210                 215                 220

Ile Val
225
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<210> 25

<211> 1187

<212> DNA

<213> Mus musculus

<220>

<221> cDNA

<222> (1)..(1187)

<220>

<221> CDS

<222> (36)..(713)

<400> 25

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ccattgtctg tactgtttct gctgccctgg aaacc atg caa gga ctg gca cag        53
                                       Met Gln Gly Leu Ala Gln
                                       1               5

acc acc atg gca gtg gtt cct gga ggt gct cca cct tca gag aat tct      101
Thr Thr Met Ala Val Val Pro Gly Gly Ala Pro Pro Ser Glu Asn Ser
            10                  15                  20

gtt ata aaa tca caa atg tgg aac aag aac aaa gag aaa ttc ttg aag      149
Val Ile Lys Ser Gln Met Trp Asn Lys Asn Lys Glu Lys Phe Leu Lys
        25                  30                  35

ggg gaa ccc aaa gtc ctc ggg gct ata caa gtt atg att gct ttc ata      197
Gly Glu Pro Lys Val Leu Gly Ala Ile Gln Val Met Ile Ala Phe Ile
    40                  45                  50

aac ttc agc tta gga ata ata att ata tta aat aga gtt tct gaa cga      245
Asn Phe Ser Leu Gly Ile Ile Ile Ile Leu Asn Arg Val Ser Glu Arg
55                  60                  65                  70

ttc atg tca gtg ctc tta ctg gcc cca ttt tgg gga tca ata atg ttc      293
Phe Met Ser Val Leu Leu Leu Ala Pro Phe Trp Gly Ser Ile Met Phe
                75                  80                  85

att ttc tca gga tcc ctg tca att gca gca gga gtg aaa cct aca aaa      341
Ile Phe Ser Gly Ser Leu Ser Ile Ala Ala Gly Val Lys Pro Thr Lys
            90                  95                  100

gcc atg atc atc agc agt cta agt gtg aac act atc agt tct gtg ttg      389
Ala Met Ile Ile Ser Ser Leu Ser Val Asn Thr Ile Ser Ser Val Leu
        105                 110                 115

gct gtg gca gca agc att att ggc gtc atc agt gtg att tct ggt gtt      437
Ala Val Ala Ala Ser Ile Ile Gly Val Ile Ser Val Ile Ser Gly Val
    120                 125                 130

ttt cgc caa ttt aga agt caa cca gcc atc gct agt ttg gat gtt ttg      485
Phe Arg Gln Phe Arg Ser Gln Pro Ala Ile Ala Ser Leu Asp Val Leu
135                 140                 145                 150

atg aca att ttg aat atg cta gaa ttc tgc att gct gtg tcc gtc tct      533
Met Thr Ile Leu Asn Met Leu Glu Phe Cys Ile Ala Val Ser Val Ser
                155                 160                 165

gca ttt ggg tgt aaa gct tcc tgt tgt aac tcc agt gag gtt ctt gta      581
Ala Phe Gly Cys Lys Ala Ser Cys Cys Asn Ser Ser Glu Val Leu Val
            170                 175                 180
```

```
gtg cta cca tca aat tct gct gtg aca gtg aca gca ccc ccc atg ata      629
Val Leu Pro Ser Asn Ser Ala Val Thr Val Thr Ala Pro Pro Met Ile
        185                     190                     195

ctt caa cca ttg cca cca tca gaa tgc caa ggg aaa aat gtt cca gaa      677
Leu Gln Pro Leu Pro Pro Ser Glu Cys Gln Gly Lys Asn Val Pro Glu
    200                     205                     210

aat cta tac agg aac caa cca gga gaa ata gtc taa ttttgatgta           723
Asn Leu Tyr Arg Asn Gln Pro Gly Glu Ile Val
215                     220                     225

cgtgttttca tgtgtgagag tgtgtgtgtg cacatgtgta tgcatttggc tttttgtacg    783

aatataagac atgctctaaa taagtaagtc aagcatttat taagtcaata acactttaaa    843

atcttctatt cattgtatgt acaagaagaa tttaattggc aaaaaaaatt ttaatgtaaa    903

ctgataaaaa ccttggttta gtgaggtgaa gtctccaaag agtgaagaat ggagttcatg    963

ggtttttttt gggggggggt tgctaaataa ataaatttgg ctgctcacga tcatatacag   1023

acacataaac tatgtaagac agcaggtgta ttaccttact aataatagca accaatttta   1083

atcctacatg aatgttcatc ttaatagtat atgccttaat tgttatggtg aaaagttaca   1143

ataaacaaaa gtgattgaaa aaaaaaaaaa aaaaaaaaaa aaag                    1187


<210>  26

<211>  225

<212>  PRT

<213>  Mus musculus


<400>  26

Met Gln Gly Leu Ala Gln Thr Thr Met Ala Val Val Pro Gly Gly Ala
1               5                   10                  15


Pro Pro Ser Glu Asn Ser Val Ile Lys Ser Gln Met Trp Asn Lys Asn
            20                  25                  30


Lys Glu Lys Phe Leu Lys Gly Glu Pro Lys Val Leu Gly Ala Ile Gln
        35                  40                  45


Val Met Ile Ala Phe Ile Asn Phe Ser Leu Gly Ile Ile Ile Ile Leu
    50                  55                  60


Asn Arg Val Ser Glu Arg Phe Met Ser Val Leu Leu Leu Ala Pro Phe
65                  70                  75                  80
```

```
Trp Gly Ser Ile Met Phe Ile Phe Ser Gly Ser Leu Ser Ile Ala Ala
                85                  90                  95

Gly Val Lys Pro Thr Lys Ala Met Ile Ile Ser Ser Leu Ser Val Asn
            100                 105                 110

Thr Ile Ser Ser Val Leu Ala Val Ala Ala Ser Ile Ile Gly Val Ile
        115                 120                 125

Ser Val Ile Ser Gly Val Phe Arg Gln Phe Arg Ser Gln Pro Ala Ile
    130                 135                 140

Ala Ser Leu Asp Val Leu Met Thr Ile Leu Asn Met Leu Glu Phe Cys
145                 150                 155                 160

Ile Ala Val Ser Val Ser Ala Phe Gly Cys Lys Ala Ser Cys Cys Asn
                165                 170                 175

Ser Ser Glu Val Leu Val Val Leu Pro Ser Asn Ser Ala Val Thr Val
            180                 185                 190

Thr Ala Pro Pro Met Ile Leu Gln Pro Leu Pro Pro Ser Glu Cys Gln
        195                 200                 205

Gly Lys Asn Val Pro Glu Asn Leu Tyr Arg Asn Gln Pro Gly Glu Ile
    210                 215                 220

Val
225
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<210> 27

<211> 1687

<212> DNA

<213> Mus musculus

<220>

<221> cDNA

<222> (1)..(1687)

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<220>

<221>  CDS

<222>  (133)..(867)


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tcgggttcct gagagctcgg ctggaagtga ctgggtgaca aggcacacag gctcagccgt     120

gggagctcaa tc atg att cca caa gta gtg acc agt gag act gtg gca atg     171
              Met Ile Pro Gln Val Val Thr Ser Glu Thr Val Ala Met
              1               5                   10

att tcg cca aat gga atg agt ctt ccc caa aca gac aaa ccc cag cct       219
Ile Ser Pro Asn Gly Met Ser Leu Pro Gln Thr Asp Lys Pro Gln Pro
    15                  20                  25

ttc cac cag tgg caa gac agc ctg aag aaa cat cta aag gct gag atc       267
Phe His Gln Trp Gln Asp Ser Leu Lys Lys His Leu Lys Ala Glu Ile
30                  35                  40                  45

aaa gtg atg gcg gca atc cag atc atg tgt gct gtg atg gtg ttg agt       315
Lys Val Met Ala Ala Ile Gln Ile Met Cys Ala Val Met Val Leu Ser
                50                  55                  60

ctg gga atc att ttg gca tct gtt ccc tcc aat cta cac ttt acc tca       363
Leu Gly Ile Ile Leu Ala Ser Val Pro Ser Asn Leu His Phe Thr Ser
            65                  70                  75

gtg ttt tca gtc ctg tta aaa tct ggc tac cca ttt ata gga gct ttg       411
Val Phe Ser Val Leu Leu Lys Ser Gly Tyr Pro Phe Ile Gly Ala Leu
        80                  85                  90

ttt ttt ata gtc tct gga att ctg tcc atc gtc acg gag aca aag tca       459
Phe Phe Ile Val Ser Gly Ile Leu Ser Ile Val Thr Glu Thr Lys Ser
    95                  100                 105

aca aaa att ttg gta gac agc agc ctg act ctg aat atc ctg agt gtt       507
Thr Lys Ile Leu Val Asp Ser Ser Leu Thr Leu Asn Ile Leu Ser Val
110                 115                 120                 125

tca ttt gct ttc atg ggc atc att atc atc tct gtc agc ctg gct ggt       555
Ser Phe Ala Phe Met Gly Ile Ile Ile Ile Ser Val Ser Leu Ala Gly
                130                 135                 140

ttg cat cct gcc tca gag cag tgc ttg cag agc aag gag ctt aga cca       603
Leu His Pro Ala Ser Glu Gln Cys Leu Gln Ser Lys Glu Leu Arg Pro
            145                 150                 155

act gaa tat cat tac tac caa ttc ttg gac agg aac gag tgc ttt gcc       651
Thr Glu Tyr His Tyr Tyr Gln Phe Leu Asp Arg Asn Glu Cys Phe Ala
        160                 165                 170

gcc aag tct gtt ctg gct gga gtc ttt tca ctg atg ctg atc agt act       699
Ala Lys Ser Val Leu Ala Gly Val Phe Ser Leu Met Leu Ile Ser Thr
    175                 180                 185
```

```
atg ttg gaa ctt ggc ctg gct gtc ctc act gcc atg ctg tgg tgg aaa      747
Met Leu Glu Leu Gly Leu Ala Val Leu Thr Ala Met Leu Trp Trp Lys
190                 195                 200                 205

cag agt cac tct aac atc cct ggg aat gtt atg ttc ctg cca cat agc      795
Gln Ser His Ser Asn Ile Pro Gly Asn Val Met Phe Leu Pro His Ser
                210                 215                 220

tca aat aat gac tcc aac atg gaa tca aag gta ctt tgt aac ccc tca      843
Ser Asn Asn Asp Ser Asn Met Glu Ser Lys Val Leu Cys Asn Pro Ser
            225                 230                 235

tat gag gaa caa ttg gtt tgt taa gaaaaacaaa acaaaacaaa actaaatacc     897
Tyr Glu Glu Gln Leu Val Cys
        240

acaaaaacaa atggaactat accgcagaag atatgtcttc atgataatgc agaaattcca     957

accatcacag ggtagcaatg cttgctactt aaaatgtaga ctgttcatac agtgggtacc    1017

agtatgagtt gaatgtgtgt attactggca ccctattgat tttcatgacc ttggcttcag    1077

ccaaagccca gacctacaaa tggtggcctt tcttagaaaa ccaaacagaa tgtttcaggc    1137

cattgtagtg gggaaaaggg acaacatttt cttgcccccct gcaattaaca gcaacagtta   1197

accattagca gtctttgtat tcagaatctc tgctatgacg tgggtcttct atcatatcag    1257

ttttattcac tggtgtgaat aaacaaggga cctgcaatga agtctgaaga agtttccatg    1317

ttgttggttc aaaataaagt ctttttgtga ttgtatattc tttttgtatg gggttttgtg    1377

tgggttttgt tgttgttgtt gttttgcttt tatgcacaga tcaagtccta agtggtacat    1437

aaacacatgt gtttgctatt ttttttttgt tctctatcaa ccaaaaaaaa aaaaaaaaaa    1497

ggcatgggaa agagaaaaag gaatatatct gtgtctctgt gtttatgtgg tgtgtgtgtg    1557

tgtgtgtttt aaactaaaac tgaatgaaca ttcaaagttt agcaatgtat tttggaggta    1617

ccagataaca tacttatttc tagaatcgcc agagcaaatg agagacattt tgtcgcttct    1677

tataagaggc                                                           1687
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<210> 28

<211> 244

<212> PRT

<213> Mus musculus

<400> 28

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Met Ile Pro Gln Val Val Thr Ser Glu Thr Val Ala Met Ile Ser Pro
1               5                   10                  15
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Asn Gly Met Ser Leu Pro Gln Thr Asp Lys Pro Gln Pro Phe His Gln
20 25 30

Trp Gln Asp Ser Leu Lys Lys His Leu Lys Ala Glu Ile Lys Val Met
35 40 45

Ala Ala Ile Gln Ile Met Cys Ala Val Met Val Leu Ser Leu Gly Ile
50 55 60

Ile Leu Ala Ser Val Pro Ser Asn Leu His Phe Thr Ser Val Phe Ser
65 70 75 80

Val Leu Leu Lys Ser Gly Tyr Pro Phe Ile Gly Ala Leu Phe Phe Ile
85 90 95

Val Ser Gly Ile Leu Ser Ile Val Thr Glu Thr Lys Ser Thr Lys Ile
100 105 110

Leu Val Asp Ser Ser Leu Thr Leu Asn Ile Leu Ser Val Ser Phe Ala
115 120 125

Phe Met Gly Ile Ile Ile Ile Ser Val Ser Leu Ala Gly Leu His Pro
130 135 140

Ala Ser Glu Gln Cys Leu Gln Ser Lys Glu Leu Arg Pro Thr Glu Tyr
145 150 155 160

His Tyr Tyr Gln Phe Leu Asp Arg Asn Glu Cys Phe Ala Ala Lys Ser
165 170 175

Val Leu Ala Gly Val Phe Ser Leu Met Leu Ile Ser Thr Met Leu Glu
180 185 190

Leu Gly Leu Ala Val Leu Thr Ala Met Leu Trp Trp Lys Gln Ser His
195 200 205

Ser Asn Ile Pro Gly Asn Val Met Phe Leu Pro His Ser Ser Asn Asn
210 215 220

Asp Ser Asn Met Glu Ser Lys Val Leu Cys Asn Pro Ser Tyr Glu Glu
225 230 235 240

Gln Leu Val Cys

<210> 29

<211> 899

<212> DNA

<213> Mus musculus

<220>

<221> cDNA

<222> (1)..(899)

<220>

<221> CDS

<222> (164)..(817)

<400> 29
aattcggcac taggaggaag ggctggagac agttacaagt tcacaagaag gaaccaagga 60

tcagcctgag agaacccaga gttaaggctc ttagggtttc tgagagctcg gctggaagtg 120

actgggtgac aaggcacaca ggctcagctg tggcagctcc atc atg att cca cag 175
Met Ile Pro Gln
1

gta gtg acc aat gag acc atc aca acg att tca cca aat gga atc aac 223
Val Val Thr Asn Glu Thr Ile Thr Thr Ile Ser Pro Asn Gly Ile Asn
5 10 15 20

ttt ccc caa aaa gac gag tcc cag cct acc caa cag agg caa gac agc 271
Phe Pro Gln Lys Asp Glu Ser Gln Pro Thr Gln Gln Arg Gln Asp Ser
25 30 35

ctg aag aaa cat cta aag gct gag atc aaa gtg ata gtg gca atc cag 319
Leu Lys Lys His Leu Lys Ala Glu Ile Lys Val Ile Val Ala Ile Gln
40 45 50

atc atg tgt gct gtg aca gtg ttg gct ctg gga atc att ttg gca tct 367
Ile Met Cys Ala Val Thr Val Leu Ala Leu Gly Ile Ile Leu Ala Ser
55 60 65

gtt cct cct gtc cca tat ttt aac tca gtg ttt tct gtc ctg tta aaa 415
Val Pro Pro Val Pro Tyr Phe Asn Ser Val Phe Ser Val Leu Leu Lys
70 75 80

tct ggc tac cca ttt ata gga gct ttg ttt ttt att gcc tct gga att 463
Ser Gly Tyr Pro Phe Ile Gly Ala Leu Phe Phe Ile Ala Ser Gly Ile
85 90 95 100

ttg tcc atc att acg gag aga aag tca aca aaa cct ttg gta gat gcc 511
Leu Ser Ile Ile Thr Glu Arg Lys Ser Thr Lys Pro Leu Val Asp Ala

```
                105                 110                 115

agc cta act ctg aat atc ctg agt gtt tca ttt gct ttc gtg ggc atc        559
Ser Leu Thr Leu Asn Ile Leu Ser Val Ser Phe Ala Phe Val Gly Ile
            120                 125                 130

att atc atc tct gtc agc ctg gct ggt ttg cat cct gcc tca gag caa        607
Ile Ile Ile Ser Val Ser Leu Ala Gly Leu His Pro Ala Ser Glu Gln
        135                 140                 145

tgc aag cag agc aag gag ctt agt cta att gaa cat gac tac tac caa        655
Cys Lys Gln Ser Lys Glu Leu Ser Leu Ile Glu His Asp Tyr Tyr Gln
    150                 155                 160

cct ttc tac aac tca gac agg agt gaa tgt gcc gtc acc aag tct att        703
Pro Phe Tyr Asn Ser Asp Arg Ser Glu Cys Ala Val Thr Lys Ser Ile
165                 170                 175                 180

ctg act gga gcc ctt tca gtg atg ctg atc atc agt gtg ttg gag ctt        751
Leu Thr Gly Ala Leu Ser Val Met Leu Ile Ile Ser Val Leu Glu Leu
                185                 190                 195

ggt ctg gct ttg ctc tct gcc atg ctg tgg ttg aga gag ggt gtt ctg        799
Gly Leu Ala Leu Leu Ser Ala Met Leu Trp Leu Arg Glu Gly Val Leu
            200                 205                 210

act tcc ctt aga atg tga tttttctgt cttataactc caataatgaa                847
Thr Ser Leu Arg Met
        215

gccaatgtgg aatcaaaggc actttgtaac tgtgcatatg aggaacgatt gt              899


<210>  30

<211>  217

<212>  PRT

<213>  Mus musculus


<400>  30

Met Ile Pro Gln Val Val Thr Asn Glu Thr Ile Thr Thr Ile Ser Pro
1               5                   10                  15

Asn Gly Ile Asn Phe Pro Gln Lys Asp Glu Ser Gln Pro Thr Gln Gln
            20                  25                  30

Arg Gln Asp Ser Leu Lys Lys His Leu Lys Ala Glu Ile Lys Val Ile
        35                  40                  45

Val Ala Ile Gln Ile Met Cys Ala Val Thr Val Leu Ala Leu Gly Ile
    50                  55                  60
```

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Ile Leu Ala Ser Val Pro Pro Val Pro Tyr Phe Asn Ser Val Phe Ser
65                  70                  75                  80

Val Leu Leu Lys Ser Gly Tyr Pro Phe Ile Gly Ala Leu Phe Phe Ile
                85                  90                  95

Ala Ser Gly Ile Leu Ser Ile Ile Thr Glu Arg Lys Ser Thr Lys Pro
            100                 105                 110

Leu Val Asp Ala Ser Leu Thr Leu Asn Ile Leu Ser Val Ser Phe Ala
        115                 120                 125

Phe Val Gly Ile Ile Ile Ile Ser Val Ser Leu Ala Gly Leu His Pro
    130                 135                 140

Ala Ser Glu Gln Cys Lys Gln Ser Lys Glu Leu Ser Leu Ile Glu His
145                 150                 155                 160

Asp Tyr Tyr Gln Pro Phe Tyr Asn Ser Asp Arg Ser Glu Cys Ala Val
                165                 170                 175

Thr Lys Ser Ile Leu Thr Gly Ala Leu Ser Val Met Leu Ile Ile Ser
            180                 185                 190

Val Leu Glu Leu Gly Leu Ala Leu Leu Ser Ala Met Leu Trp Leu Arg
        195                 200                 205

Glu Gly Val Leu Thr Ser Leu Arg Met
    210                 215
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<210> 31

<211> 1370

<212> DNA

<213> Mus musculus

<220>

<221> cDNA

<222> (1)..(1370)

<220>

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<221>  CDS

<222>  (110)..(853)


<400>  31
caaggatcag actgagaaaa cccatagtta aagctcttgg ggtttctgag agctcagctg      60

gaagtgactg ggtgacaagg cgcacaggct cagccgtgga agctccatc atg att cca     118
                                                      Met Ile Pro
                                                      1

caa gta gtg acc agt gag act gtc aca gtg att tca cca aat gga atc      166
Gln Val Val Thr Ser Glu Thr Val Thr Val Ile Ser Pro Asn Gly Ile
    5                   10                  15

agc ttt ccc caa aca gac aaa ccc cag cct tcc cac cag agc caa gac      214
Ser Phe Pro Gln Thr Asp Lys Pro Gln Pro Ser His Gln Ser Gln Asp
20                  25                  30                  35

agc ctg aag aaa cat cta aag gct gag atc aaa gtg atg gcg gca atc      262
Ser Leu Lys Lys His Leu Lys Ala Glu Ile Lys Val Met Ala Ala Ile
                40                  45                  50

cag atc atg tgt gct gtg atg gtg ttg agt ctg gga atc att ttg gca      310
Gln Ile Met Cys Ala Val Met Val Leu Ser Leu Gly Ile Ile Leu Ala
            55                  60                  65

tct gtt ccc tcc aat cta cac ttt acc tca gtg ttt tcc atc ctg tta      358
Ser Val Pro Ser Asn Leu His Phe Thr Ser Val Phe Ser Ile Leu Leu
        70                  75                  80

gaa tct ggc tac cca ttt gta gga gct ttg ttt ttt gcc atc tct gga      406
Glu Ser Gly Tyr Pro Phe Val Gly Ala Leu Phe Phe Ala Ile Ser Gly
    85                  90                  95

att ctg tct att gtc aca gag aaa aag atg act aag cct ttg gtt cac      454
Ile Leu Ser Ile Val Thr Glu Lys Lys Met Thr Lys Pro Leu Val His
100                 105                 110                 115

agc agc cta gcc ctg agc atc ctg agt gtc ctc tct gct ctt aca ggc      502
Ser Ser Leu Ala Leu Ser Ile Leu Ser Val Leu Ser Ala Leu Thr Gly
                120                 125                 130

atc gct att ctc tct gtc agt ttg gct gct tta gag cct gcc ttg cag      550
Ile Ala Ile Leu Ser Val Ser Leu Ala Ala Leu Glu Pro Ala Leu Gln
            135                 140                 145

caa tgt aag ctg gct ttc aca caa cta gac aca acc caa gac gct tat      598
Gln Cys Lys Leu Ala Phe Thr Gln Leu Asp Thr Thr Gln Asp Ala Tyr
        150                 155                 160

cat ttc ttt agc cct gag cca tta aac agc tgc ttc gtg gcc aaa gct      646
His Phe Phe Ser Pro Glu Pro Leu Asn Ser Cys Phe Val Ala Lys Ala
    165                 170                 175

gct ctg act gga gtc ttt tca ctg atg cta atc agc agt gtg ttg gag      694
Ala Leu Thr Gly Val Phe Ser Leu Met Leu Ile Ser Ser Val Leu Glu
180                 185                 190                 195
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ctt ggc ctg gct gtc ctc act gcc aca ctg tgg tgg aaa cag agc tcc      742
Leu Gly Leu Ala Val Leu Thr Ala Thr Leu Trp Trp Lys Gln Ser Ser
                200                 205                 210

tct gct ttc tct ggg aat gtg att ttc ctg tct cag aac tca aag aat      790
Ser Ala Phe Ser Gly Asn Val Ile Phe Leu Ser Gln Asn Ser Lys Asn
            215                 220                 225

aaa tcc agt gta tct tca gag tca ctt tgt aac cct aca tat gaa aac      838
Lys Ser Ser Val Ser Ser Glu Ser Leu Cys Asn Pro Thr Tyr Glu Asn
        230                 235                 240

ata ttg act tca taa gaattaagta gaggttatat agcagaaaaa tctgtcttta      893
Ile Leu Thr Ser
    245

acatgattta gaaaagccat ttactgtgtg acaacaatgc ttaatatctt gatatcttaa    953

tgtgtgtatt ggttaatcag caaccatgaa aaacatacta actggctggg ttcagtagca   1013

cgctcttgat ttggtgtcag tcaaaacaca gacctgtaaa ttccaattta tgtagtggtc   1073

aaagagcccc aattattttc tcaaaaaact ggaagaatgt ttcataggat catggtggag   1133

ccaatgggca acagttcttc ttatccttgt cacttggctg caggaggtac tgactagggc   1193

ctgagatcat attctgtgtg cgtggcatgg acttcatggc atctatttta ttcataagca   1253

catgaaaaca agtcatctct tatgaagtct caaagagcat aaaaaagtta gcctccaaat   1313

aaagtcttta tataatcccc ccaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaa      1370


<210>  32

<211>  247

<212>  PRT

<213>  Mus musculus


<400>  32

Met Ile Pro Gln Val Val Thr Ser Glu Thr Val Thr Val Ile Ser Pro
1               5                   10                  15


Asn Gly Ile Ser Phe Pro Gln Thr Asp Lys Pro Gln Pro Ser His Gln
            20                  25                  30


Ser Gln Asp Ser Leu Lys Lys His Leu Lys Ala Glu Ile Lys Val Met
        35                  40                  45


Ala Ala Ile Gln Ile Met Cys Ala Val Met Val Leu Ser Leu Gly Ile
    50                  55                  60
```

```
Ile Leu Ala Ser Val Pro Ser Asn Leu His Phe Thr Ser Val Phe Ser
65                  70                  75                  80

Ile Leu Leu Glu Ser Gly Tyr Pro Phe Val Gly Ala Leu Phe Phe Ala
                85                  90                  95

Ile Ser Gly Ile Leu Ser Ile Val Thr Glu Lys Lys Met Thr Lys Pro
            100                 105                 110

Leu Val His Ser Ser Leu Ala Leu Ser Ile Leu Ser Val Leu Ser Ala
        115                 120                 125

Leu Thr Gly Ile Ala Ile Leu Ser Val Ser Leu Ala Ala Leu Glu Pro
    130                 135                 140

Ala Leu Gln Gln Cys Lys Leu Ala Phe Thr Gln Leu Asp Thr Thr Gln
145                 150                 155                 160

Asp Ala Tyr His Phe Phe Ser Pro Glu Pro Leu Asn Ser Cys Phe Val
                165                 170                 175

Ala Lys Ala Ala Leu Thr Gly Val Phe Ser Leu Met Leu Ile Ser Ser
            180                 185                 190

Val Leu Glu Leu Gly Leu Ala Val Leu Thr Ala Thr Leu Trp Trp Lys
        195                 200                 205

Gln Ser Ser Ser Ala Phe Ser Gly Asn Val Ile Phe Leu Ser Gln Asn
    210                 215                 220

Ser Lys Asn Lys Ser Ser Val Ser Ser Glu Ser Leu Cys Asn Pro Thr
225                 230                 235                 240

Tyr Glu Asn Ile Leu Thr Ser
                245
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<210> 33

<211> 938

<212> DNA

<213> Mus musculus

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<220>

<221>  cDNA

<222>  (1)..(938)


<220>

<221>  CDS

<222>  (105)..(809)


<400>  33
aacagctggt aagaggttga gtctgagttc cccaggagca gagtgagcag gcccaggagc      60

tgcagcacac aggacagggc tgtgaggaca gcatccctgg cacc atg cgt cta cag      116
                                                 Met Arg Leu Gln
                                                 1

ctt ggc acc aag aac att ggg tgg gac tgc ttt cca aag gac atc att      164
Leu Gly Thr Lys Asn Ile Gly Trp Asp Cys Phe Pro Lys Asp Ile Ile
5                   10                  15                  20

atc cac aaa aga gag aaa act gga cat aca tat gaa aaa gaa gat gac      212
Ile His Lys Arg Glu Lys Thr Gly His Thr Tyr Glu Lys Glu Asp Asp
                25                  30                  35

ctg ctg att gga gtg cct agt gaa gcc aca ctt ctt gga acc atc cag      260
Leu Leu Ile Gly Val Pro Ser Glu Ala Thr Leu Leu Gly Thr Ile Gln
            40                  45                  50

cta cta tgt gcc ctg ata ctt gca agc ttt ggg ggc att ttg gtg tca      308
Leu Leu Cys Ala Leu Ile Leu Ala Ser Phe Gly Gly Ile Leu Val Ser
        55                  60                  65

gct tcc tac ttc aac cca gaa gtt tcc acc act ttg ata tct gga tac      356
Ala Ser Tyr Phe Asn Pro Glu Val Ser Thr Thr Leu Ile Ser Gly Tyr
    70                  75                  80

ctg ttt att gga tcc ctc tgt ttt gcc att gct gga att ctc tcc att      404
Leu Phe Ile Gly Ser Leu Cys Phe Ala Ile Ala Gly Ile Leu Ser Ile
85                  90                  95                  100

atc tct gaa aaa atc tca acc aaa ccc ttt gca ctg agc agc ctg gcc      452
Ile Ser Glu Lys Ile Ser Thr Lys Pro Phe Ala Leu Ser Ser Leu Ala
                105                 110                 115

tcc aat gta gca agc tct gtt gtt gcc gtc att ggc ctc ttc ctc ttc      500
Ser Asn Val Ala Ser Ser Val Val Ala Val Ile Gly Leu Phe Leu Phe
            120                 125                 130

acc tat tgt ctg ata gcc ctg ggg agt gct ttc cca cac tgt aac tca      548
Thr Tyr Cys Leu Ile Ala Leu Gly Ser Ala Phe Pro His Cys Asn Ser
        135                 140                 145

gaa aag aaa ttc ctg tcc tta ttg tct tac ttg aaa tcc cac cat tgg      596
Glu Lys Lys Phe Leu Ser Leu Leu Ser Tyr Leu Lys Ser His His Trp
```

```
    150                 155                 160

aag aat gaa gac aag aac tgt tac ctg gct tat gtc ggt gct atg agt        644
Lys Asn Glu Asp Lys Asn Cys Tyr Leu Ala Tyr Val Gly Ala Met Ser
165                 170                 175                 180

gct ctg ggg atg atg ctt ctc ttc act gtg ctg gag gtt ttc cta gct        692
Ala Leu Gly Met Met Leu Leu Phe Thr Val Leu Glu Val Phe Leu Ala
                185                 190                 195

gga tac agt tct atc ttt tgg tgg aag caa gtc tac tcc aac aaa cct        740
Gly Tyr Ser Ser Ile Phe Trp Trp Lys Gln Val Tyr Ser Asn Lys Pro
            200                 205                 210

ggg ggt aca ttt ttc ttg cct caa tca caa gat cat acc caa ctt gtc        788
Gly Gly Thr Phe Phe Leu Pro Gln Ser Gln Asp His Thr Gln Leu Val
        215                 220                 225

aaa agc aat ttg ttg caa taa tggatatgaa ataaaaaaga agaaagaaga           839
Lys Ser Asn Leu Leu Gln
    230

aatctgttgc tcacagcaga gtacagctag acttgcctga aatctcagct ttggtagatc      899

ctaataaaca tctgaagaaa aaaaaaaaaa aaaaaaaaa                             938


<210>  34

<211>  234

<212>  PRT

<213>  Mus musculus


<400>  34

Met Arg Leu Gln Leu Gly Thr Lys Asn Ile Gly Trp Asp Cys Phe Pro
1               5                   10                  15


Lys Asp Ile Ile Ile His Lys Arg Glu Lys Thr Gly His Thr Tyr Glu
            20                  25                  30


Lys Glu Asp Asp Leu Leu Ile Gly Val Pro Ser Glu Ala Thr Leu Leu
        35                  40                  45


Gly Thr Ile Gln Leu Leu Cys Ala Leu Ile Leu Ala Ser Phe Gly Gly
    50                  55                  60


Ile Leu Val Ser Ala Ser Tyr Phe Asn Pro Glu Val Ser Thr Thr Leu
65                  70                  75                  80


Ile Ser Gly Tyr Leu Phe Ile Gly Ser Leu Cys Phe Ala Ile Ala Gly
```

```
            85                  90                  95

Ile Leu Ser Ile Ile Ser Glu Lys Ile Ser Thr Lys Pro Phe Ala Leu
            100                 105                 110

Ser Ser Leu Ala Ser Asn Val Ala Ser Ser Val Val Ala Val Ile Gly
        115                 120                 125

Leu Phe Leu Phe Thr Tyr Cys Leu Ile Ala Leu Gly Ser Ala Phe Pro
    130                 135                 140

His Cys Asn Ser Glu Lys Lys Phe Leu Ser Leu Leu Ser Tyr Leu Lys
145                 150                 155                 160

Ser His His Trp Lys Asn Glu Asp Lys Asn Cys Tyr Leu Ala Tyr Val
                165                 170                 175

Gly Ala Met Ser Ala Leu Gly Met Met Leu Leu Phe Thr Val Leu Glu
            180                 185                 190

Val Phe Leu Ala Gly Tyr Ser Ser Ile Phe Trp Trp Lys Gln Val Tyr
        195                 200                 205

Ser Asn Lys Pro Gly Gly Thr Phe Phe Leu Pro Gln Ser Gln Asp His
    210                 215                 220

Thr Gln Leu Val Lys Ser Asn Leu Leu Gln
225                 230
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<210> 35

<211> 1025

<212> DNA

<213> Mus musculus

<220>

<221> cDNA

<222> (1)..(1025)

<220>

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<221>  CDS

<222>  (12)..(818)


<400>  35
aattcgtcga c atg gaa ccg gag caa gag aga ctt act tgg caa ccg ggc      50
             Met Glu Pro Glu Gln Glu Arg Leu Thr Trp Gln Pro Gly
             1               5                   10

aca gta agc atg aat aca gta act tca ccg ggc ccc atg gcc aat tct       98
Thr Val Ser Met Asn Thr Val Thr Ser Pro Gly Pro Met Ala Asn Ser
    15                  20                  25

gta tat gtg gtg gca ccg ccc aac agt tat cct gtg gtc cca gga acc      146
Val Tyr Val Val Ala Pro Pro Asn Ser Tyr Pro Val Val Pro Gly Thr
30                  35                  40                  45

gtg cct cag atg cct att tat ccc agc aat cag cct caa gtc cat gtg      194
Val Pro Gln Met Pro Ile Tyr Pro Ser Asn Gln Pro Gln Val His Val
                50                  55                  60

att tct ggt cat ctg cct ggt ttg gtg cca gct atg acc gaa cca cct      242
Ile Ser Gly His Leu Pro Gly Leu Val Pro Ala Met Thr Glu Pro Pro
            65                  70                  75

gcc cag aga gtc ttg aaa aag gga caa gtc cta ggg gcc atc cag atc      290
Ala Gln Arg Val Leu Lys Lys Gly Gln Val Leu Gly Ala Ile Gln Ile
        80                  85                  90

ctg atc ggc ctg gtg cac atc ggc ctg ggc tcc atc atg atc acc aac      338
Leu Ile Gly Leu Val His Ile Gly Leu Gly Ser Ile Met Ile Thr Asn
    95                  100                 105

ctc ttc agc cac tat aca cct gtc tcc ctc tat gga ggt ttc ccc ttc      386
Leu Phe Ser His Tyr Thr Pro Val Ser Leu Tyr Gly Gly Phe Pro Phe
110                 115                 120                 125

tgg gga ggc atc tgg ttt atc att tca gga tct ctc tca gtg gcg gca      434
Trp Gly Gly Ile Trp Phe Ile Ile Ser Gly Ser Leu Ser Val Ala Ala
                130                 135                 140

gaa act cag ccc aat tca cct tgc ctg ctg aat ggc agt gtg ggc ttg      482
Glu Thr Gln Pro Asn Ser Pro Cys Leu Leu Asn Gly Ser Val Gly Leu
            145                 150                 155

aac atc ttc agc gcc atc tgt tct gcg gtg ggc atc atg ctc ttc atc      530
Asn Ile Phe Ser Ala Ile Cys Ser Ala Val Gly Ile Met Leu Phe Ile
        160                 165                 170

aca gac atc agt atc tca tca gga tac atc tac cct agc tac tac cct      578
Thr Asp Ile Ser Ile Ser Ser Gly Tyr Ile Tyr Pro Ser Tyr Tyr Pro
    175                 180                 185

tac cag gag aac ttg ggt gtg aga aca ggc gtg gct att tct agt gtg      626
Tyr Gln Glu Asn Leu Gly Val Arg Thr Gly Val Ala Ile Ser Ser Val
190                 195                 200                 205

cta ctc atc ttc tgc ctc ttg gag ctc agc att gca agt gtg tcc tcc      674
Leu Leu Ile Phe Cys Leu Leu Glu Leu Ser Ile Ala Ser Val Ser Ser
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```
                210                     215                     220

cac ttt ggc tgc caa gtg gcc tgt tgt cac tac aac aat cca ggt gtg      722
His Phe Gly Cys Gln Val Ala Cys Cys His Tyr Asn Asn Pro Gly Val
            225                     230                     235

gtc att cca aat gtc tat gca gca aac cca gtg gtc atc cca gaa cca      770
Val Ile Pro Asn Val Tyr Ala Ala Asn Pro Val Val Ile Pro Glu Pro
        240                     245                     250

cca aac cca ata cca agt tat tcc gaa gta gtt caa gac tcc aga taa      818
Pro Asn Pro Ile Pro Ser Tyr Ser Glu Val Val Gln Asp Ser Arg
    255                     260                     265

gtgaacctga agattctaga gaccaagtga catcctctcc tacctagact cctataaacc    878

aagttcttcc tttcctgacg aagggtaaat atctttcttg tggcctaaat tatagactct    938

tgcttcaact caccctggaa aaatctctat taaaacgaga tgggagattg aaatggattc    998

aaataaagat gctctagccg gaaaaaa                                        1025


<210>  36

<211>  268

<212>  PRT

<213>  Mus musculus


<400>  36

Met Glu Pro Glu Gln Glu Arg Leu Thr Trp Gln Pro Gly Thr Val Ser
1               5                   10                  15

Met Asn Thr Val Thr Ser Pro Gly Pro Met Ala Asn Ser Val Tyr Val
            20                  25                  30

Val Ala Pro Pro Asn Ser Tyr Pro Val Val Pro Gly Thr Val Pro Gln
        35                  40                  45

Met Pro Ile Tyr Pro Ser Asn Gln Pro Gln Val His Val Ile Ser Gly
    50                  55                  60

His Leu Pro Gly Leu Val Pro Ala Met Thr Glu Pro Pro Ala Gln Arg
65                  70                  75                  80

Val Leu Lys Lys Gly Gln Val Leu Gly Ala Ile Gln Ile Leu Ile Gly
                85                  90                  95

Leu Val His Ile Gly Leu Gly Ser Ile Met Ile Thr Asn Leu Phe Ser
            100                 105                 110
```

His Tyr Thr Pro Val Ser Leu Tyr Gly Gly Phe Pro Phe Trp Gly Gly
115 120 125

Ile Trp Phe Ile Ile Ser Gly Ser Leu Ser Val Ala Ala Glu Thr Gln
130 135 140

Pro Asn Ser Pro Cys Leu Leu Asn Gly Ser Val Gly Leu Asn Ile Phe
145 150 155 160

Ser Ala Ile Cys Ser Ala Val Gly Ile Met Leu Phe Ile Thr Asp Ile
165 170 175

Ser Ile Ser Ser Gly Tyr Ile Tyr Pro Ser Tyr Tyr Pro Tyr Gln Glu
180 185 190

Asn Leu Gly Val Arg Thr Gly Val Ala Ile Ser Ser Val Leu Leu Ile
195 200 205

Phe Cys Leu Leu Glu Leu Ser Ile Ala Ser Val Ser Ser His Phe Gly
210 215 220

Cys Gln Val Ala Cys Cys His Tyr Asn Asn Pro Gly Val Val Ile Pro
225 230 235 240

Asn Val Tyr Ala Ala Asn Pro Val Val Ile Pro Glu Pro Pro Asn Pro
245 250 255

Ile Pro Ser Tyr Ser Glu Val Val Gln Asp Ser Arg
260 265

<210> 37

<211> 1038

<212> DNA

<213> Mus musculus

<220>

<221> cDNA

<222> (1)..(1038)

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<220>

<221>  CDS

<222>  (75)..(878)


<400>  37
gctctggatg gtcaggcaca gctgaagcct acaacagagt tcccggggcc agccagggtt      60

cccggccagg gtca atg gct ggc caa gct ccc aca gcg gtt ccc ggc agt       110
                Met Ala Gly Gln Ala Pro Thr Ala Val Pro Gly Ser
                1               5                   10

gtt act gga gaa gtc tca cga tgg cag aac cta gga cct gcc cag cct       158
Val Thr Gly Glu Val Ser Arg Trp Gln Asn Leu Gly Pro Ala Gln Pro
        15                  20                  25

gca cag aaa gta gcc cag ccc caa aac ctg gtt cca gat ggg cac ctt       206
Ala Gln Lys Val Ala Gln Pro Gln Asn Leu Val Pro Asp Gly His Leu
    30                  35                  40

gag aaa gcc ctg gag gga agt gac ctt cta cag aag ttg ggt ggc ttt       254
Glu Lys Ala Leu Glu Gly Ser Asp Leu Leu Gln Lys Leu Gly Gly Phe
45                  50                  55                  60

cac atc gcc att gct ttt gca cac ttg gcc ttt ggt ggc tac ttg atc       302
His Ile Ala Ile Ala Phe Ala His Leu Ala Phe Gly Gly Tyr Leu Ile
                65                  70                  75

tcc aca gtc aag aat ctg cac ctg gtg gtg ttg aag tgc tgg tat cca       350
Ser Thr Val Lys Asn Leu His Leu Val Val Leu Lys Cys Trp Tyr Pro
            80                  85                  90

ctc tgg gga act gtc tct ttt ctc gtt gca ggg atg gcg gcc atg aca       398
Leu Trp Gly Thr Val Ser Phe Leu Val Ala Gly Met Ala Ala Met Thr
        95                  100                 105


aca gtg aca ttc cct aag acc tct ctg aag gtt ctc tgt gtg ata gcc       446
Thr Val Thr Phe Pro Lys Thr Ser Leu Lys Val Leu Cys Val Ile Ala
    110                 115                 120

aac gtt atc agc ttg ttc tgc gca ctg gcc ggc ttc ttt gtc att gcc       494
Asn Val Ile Ser Leu Phe Cys Ala Leu Ala Gly Phe Phe Val Ile Ala
125                 130                 135                 140

aag gac ctc ttc ctg gag ggt cct ttt cca tgg cca atc tgg aga cca       542
Lys Asp Leu Phe Leu Glu Gly Pro Phe Pro Trp Pro Ile Trp Arg Pro
                145                 150                 155

tac cct gaa ccc aca acc tac atc caa agg cta gag ctg acc ctg ttc       590
Tyr Pro Glu Pro Thr Thr Tyr Ile Gln Arg Leu Glu Leu Thr Leu Phe
            160                 165                 170

tgc ttc acc ttc ctg gag atc ttc ctg tcg ggg tcc aca gcc atc aca       638
Cys Phe Thr Phe Leu Glu Ile Phe Leu Ser Gly Ser Thr Ala Ile Thr
        175                 180                 185

gcc tat agg atg aaa cgc ctg caa gca gag gac aag gat gac acc cct       686
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Ala Tyr Arg Met Lys Arg Leu Gln Ala Glu Asp Lys Asp Asp Thr Pro
    190                 195                 200

ttc gtt cct gac aca cct atg gaa ctt aaa ggt cta tcg ctg ggg cca      734
Phe Val Pro Asp Thr Pro Met Glu Leu Lys Gly Leu Ser Leu Gly Pro
205                 210                 215                 220

cca cca tcc tat aaa gat gtg gcc caa gga cac tcc tcc tct gat act      782
Pro Pro Ser Tyr Lys Asp Val Ala Gln Gly His Ser Ser Ser Asp Thr
                225                 230                 235

ggc aga gcc ttg gcc acc agc tct gga ctg ctg ctg gcc tct gac tcc      830
Gly Arg Ala Leu Ala Thr Ser Ser Gly Leu Leu Leu Ala Ser Asp Ser
            240                 245                 250

ttc cat caa gct ctg ctt cac acg ggc cca agg acc ttg aga aag tag      878
Phe His Gln Ala Leu Leu His Thr Gly Pro Arg Thr Leu Arg Lys
        255                 260                 265

tctaagacta ctagactttc ccaataaaga ctgagttcct gcaatgccaa gtccttccct    938

gcctcccatt ggctggactc cggtgatgac acaggcgtga cttgcacaaa taaatgcata    998

accactatga aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa                         1038


<210>  38

<211>  267

<212>  PRT

<213>  Mus musculus


<400>  38

Met Ala Gly Gln Ala Pro Thr Ala Val Pro Gly Ser Val Thr Gly Glu
1               5                   10                  15

Val Ser Arg Trp Gln Asn Leu Gly Pro Ala Gln Pro Ala Gln Lys Val
            20                  25                  30

Ala Gln Pro Gln Asn Leu Val Pro Asp Gly His Leu Glu Lys Ala Leu
        35                  40                  45

Glu Gly Ser Asp Leu Leu Gln Lys Leu Gly Gly Phe His Ile Ala Ile
    50                  55                  60

Ala Phe Ala His Leu Ala Phe Gly Gly Tyr Leu Ile Ser Thr Val Lys
65                  70                  75                  80

Asn Leu His Leu Val Val Leu Lys Cys Trp Tyr Pro Leu Trp Gly Thr
                85                  90                  95
```

```
Val Ser Phe Leu Val Ala Gly Met Ala Ala Met Thr Thr Val Thr Phe
            100                 105                 110

Pro Lys Thr Ser Leu Lys Val Leu Cys Val Ile Ala Asn Val Ile Ser
        115                 120                 125

Leu Phe Cys Ala Leu Ala Gly Phe Phe Val Ile Ala Lys Asp Leu Phe
    130                 135                 140

Leu Glu Gly Pro Phe Pro Trp Pro Ile Trp Arg Pro Tyr Pro Glu Pro
145                 150                 155                 160

Thr Thr Tyr Ile Gln Arg Leu Glu Leu Thr Leu Phe Cys Phe Thr Phe
                165                 170                 175

Leu Glu Ile Phe Leu Ser Gly Ser Thr Ala Ile Thr Ala Tyr Arg Met
            180                 185                 190

Lys Arg Leu Gln Ala Glu Asp Lys Asp Asp Thr Pro Phe Val Pro Asp
        195                 200                 205

Thr Pro Met Glu Leu Lys Gly Leu Ser Leu Gly Pro Pro Pro Ser Tyr
    210                 215                 220

Lys Asp Val Ala Gln Gly His Ser Ser Ser Asp Thr Gly Arg Ala Leu
225                 230                 235                 240

Ala Thr Ser Ser Gly Leu Leu Leu Ala Ser Asp Ser Phe His Gln Ala
                245                 250                 255

Leu Leu His Thr Gly Pro Arg Thr Leu Arg Lys
            260                 265
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<210> 39

<211> 1146

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<213> Homo sapiens

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<222> (134)..(1027)

<300>

<301> Tedder, TF, Klejman, G, Schlossman, SF, Saito, H

<302> Structure of the gene encoding the human B lymphocyte differentiation antigen CD20 (B1)

<303> J Immunol

<304> 142

<305> 7

<306> 2560-2568

<307> 1989-__-__

<309> ____-__-__

<300>

<301> Tedder, TF, Streuli, M, Schlossman, SF, and Saito H

<302> Isolation and structure of a cDNA encoding the B1 (CD20) cell-surface antigen of human B lymphocytes

<303> Proc Natl Acad Sci USA

<304> 85

<305> 1

<306> 208-212

<307> 1988-__-__

<309> ____-__-__

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agactgccaa aaatcttgtt cttgctctcc tcattttgtt atttgtttta tttttaggag     120

ttttgagagc aaa atg aca aca ccc aga aat tca gta aat ggg act ttc        169
               Met Thr Thr Pro Arg Asn Ser Val Asn Gly Thr Phe
               1               5                   10

ccg gca gag cca atg aaa ggc cct att gct atg caa tct ggt cca aaa       217
Pro Ala Glu Pro Met Lys Gly Pro Ile Ala Met Gln Ser Gly Pro Lys
```

15 20 25

cca ctc ttc agg agg atg tct tca ctg gtg ggc ccc acg caa agc ttc 265
Pro Leu Phe Arg Arg Met Ser Ser Leu Val Gly Pro Thr Gln Ser Phe
30 35 40

ttc atg agg gaa tct aag act ttg ggg gct gtc cag att atg aat ggg 313
Phe Met Arg Glu Ser Lys Thr Leu Gly Ala Val Gln Ile Met Asn Gly
45 50 55 60

ctc ttc cac att gcc ctg ggg ggt ctt ctg atg atc cca gca ggg atc 361
Leu Phe His Ile Ala Leu Gly Gly Leu Leu Met Ile Pro Ala Gly Ile
65 70 75

tat gca ccc atc tgt gtg act gtg tgg tac cct ctc tgg gga ggc att 409
Tyr Ala Pro Ile Cys Val Thr Val Trp Tyr Pro Leu Trp Gly Gly Ile
80 85 90

atg tat att att tcc gga tca ctc ttg gca gca acg gag aaa aac tct 457
Met Tyr Ile Ile Ser Gly Ser Leu Leu Ala Ala Thr Glu Lys Asn Ser
95 100 105

agg aag tgt ttg gtc aaa gga aaa atg ata atg aat tca ttg agc ctc 505
Arg Lys Cys Leu Val Lys Gly Lys Met Ile Met Asn Ser Leu Ser Leu
110 115 120

ttt gct gcc att tct gga atg att ctt tca atc atg gac ata ctt aat 553
Phe Ala Ala Ile Ser Gly Met Ile Leu Ser Ile Met Asp Ile Leu Asn
125 130 135 140

att aaa att tcc cat ttt tta aaa atg gag agt ctg aat ttt att aga 601
Ile Lys Ile Ser His Phe Leu Lys Met Glu Ser Leu Asn Phe Ile Arg
145 150 155

gct cac aca cca tat att aac ata tac aac tgt gaa cca gct aat ccc 649
Ala His Thr Pro Tyr Ile Asn Ile Tyr Asn Cys Glu Pro Ala Asn Pro
160 165 170

tct gag aaa aac tcc cca tct acc caa tac tgt tac agc ata caa tct 697
Ser Glu Lys Asn Ser Pro Ser Thr Gln Tyr Cys Tyr Ser Ile Gln Ser
175 180 185

ctg ttc ttg ggc att ttg tca gtg atg ctg atc ttt gcc ttc ttc cag 745
Leu Phe Leu Gly Ile Leu Ser Val Met Leu Ile Phe Ala Phe Phe Gln
190 195 200

gaa ctt gta ata gct ggc atc gtt gag aat gaa tgg aaa aga acg tgc 793
Glu Leu Val Ile Ala Gly Ile Val Glu Asn Glu Trp Lys Arg Thr Cys
205 210 215 220

tcc aga ccc aaa tct aac ata gtt ctc ctg tca gca gaa gaa aaa aaa 841
Ser Arg Pro Lys Ser Asn Ile Val Leu Leu Ser Ala Glu Glu Lys Lys
225 230 235

gaa cag act att gaa ata aaa gaa gaa gtg gtt ggg cta act gaa aca 889
Glu Gln Thr Ile Glu Ile Lys Glu Glu Val Val Gly Leu Thr Glu Thr
240 245 250

tct tcc caa cca aag aat gaa gaa gac att gaa att att cca atc caa 937

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Ser Ser Gln Pro Lys Asn Glu Glu Asp Ile Glu Ile Ile Pro Ile Gln
        255                 260                 265

gaa gag gaa gaa gaa gaa aca gag acg aac ttt cca gaa cct ccc caa      985
Glu Glu Glu Glu Glu Glu Thr Glu Thr Asn Phe Pro Glu Pro Pro Gln
    270                 275                 280

gat cag gaa tcc tca cca ata gaa aat gac agc tct cct taa              1027
Asp Gln Glu Ser Ser Pro Ile Glu Asn Asp Ser Ser Pro
285                 290                 295

gtgatttctt ctgttttctg tttccttttt taaacattag tgttcatagc ttccaagaga   1087

catgctgact ttcatttctt gaggtactct gcacatacgc accacatctc tatctggcc    1146


<210>  40

<211>  297

<212>  PRT

<213>  Homo sapiens


<400>  40

Met Thr Thr Pro Arg Asn Ser Val Asn Gly Thr Phe Pro Ala Glu Pro
1               5                   10                  15

Met Lys Gly Pro Ile Ala Met Gln Ser Gly Pro Lys Pro Leu Phe Arg
            20                  25                  30

Arg Met Ser Ser Leu Val Gly Pro Thr Gln Ser Phe Phe Met Arg Glu
        35                  40                  45

Ser Lys Thr Leu Gly Ala Val Gln Ile Met Asn Gly Leu Phe His Ile
    50                  55                  60

Ala Leu Gly Gly Leu Leu Met Ile Pro Ala Gly Ile Tyr Ala Pro Ile
65                  70                  75                  80

Cys Val Thr Val Trp Tyr Pro Leu Trp Gly Gly Ile Met Tyr Ile Ile
                85                  90                  95

Ser Gly Ser Leu Leu Ala Ala Thr Glu Lys Asn Ser Arg Lys Cys Leu
            100                 105                 110

Val Lys Gly Lys Met Ile Met Asn Ser Leu Ser Leu Phe Ala Ala Ile
        115                 120                 125

Ser Gly Met Ile Leu Ser Ile Met Asp Ile Leu Asn Ile Lys Ile Ser
```

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        130                 135                 140

His Phe Leu Lys Met Glu Ser Leu Asn Phe Ile Arg Ala His Thr Pro
145                 150                 155                 160

Tyr Ile Asn Ile Tyr Asn Cys Glu Pro Ala Asn Pro Ser Glu Lys Asn
                165                 170                 175

Ser Pro Ser Thr Gln Tyr Cys Tyr Ser Ile Gln Ser Leu Phe Leu Gly
            180                 185                 190

Ile Leu Ser Val Met Leu Ile Phe Ala Phe Phe Gln Glu Leu Val Ile
        195                 200                 205

Ala Gly Ile Val Glu Asn Glu Trp Lys Arg Thr Cys Ser Arg Pro Lys
    210                 215                 220

Ser Asn Ile Val Leu Leu Ser Ala Glu Glu Lys Lys Glu Gln Thr Ile
225                 230                 235                 240

Glu Ile Lys Glu Glu Val Val Gly Leu Thr Glu Thr Ser Ser Gln Pro
                245                 250                 255

Lys Asn Glu Glu Asp Ile Glu Ile Ile Pro Ile Gln Glu Glu Glu Glu
            260                 265                 270

Glu Glu Thr Glu Thr Asn Phe Pro Glu Pro Pro Gln Asp Gln Glu Ser
        275                 280                 285

Ser Pro Ile Glu Asn Asp Ser Ser Pro
    290                 295
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<210> 41

<211> 894

<212> DNA

<213> Homo sapiens

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<222> (51)..(785)

<300>

<301> Kuster, H, Zhang, L, Brini, AT, MacGlashan, DWJ, Kinet, J-P

<302> The gene and cDNA for the human high affinity immunoglobulin E receptor beta chain and expression of the complete human receptor

<303> J Biol Cham

<304> 267

<305> 18

<306> 12782-12787

<307> 1992-__-__

<309> _____-__-__

<400> 41

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tctcaatata ataatattct ttattcctgg acagctcggt taatgaaaaa atg gac         56
                                                       Met Asp
                                                       1

aca gaa agt aat agg aga gca aat ctt gct ctc cca cag gag cct tcc       104
Thr Glu Ser Asn Arg Arg Ala Asn Leu Ala Leu Pro Gln Glu Pro Ser
        5                   10                  15

agt gtg cct gca ttt gaa gtc ttg gaa ata tct ccc cag gaa gta tct       152
Ser Val Pro Ala Phe Glu Val Leu Glu Ile Ser Pro Gln Glu Val Ser
    20                  25                  30

tca ggc aga cta ttg aag tcg gcc tca tcc cca cca ctg cat aca tgg       200
Ser Gly Arg Leu Leu Lys Ser Ala Ser Ser Pro Pro Leu His Thr Trp
35                  40                  45                  50

ctg aca gtt ttg aaa aaa gag cag gag ttc ctg ggg gta aca caa att       248
Leu Thr Val Leu Lys Lys Glu Gln Glu Phe Leu Gly Val Thr Gln Ile
                55                  60                  65

ctg act gct atg ata tgc ctt tgt ttt gga aca gtt gtc tgc tct gta       296
Leu Thr Ala Met Ile Cys Leu Cys Phe Gly Thr Val Val Cys Ser Val
            70                  75                  80

ctt gat att tca cac att gag gga gac att ttt tca tca ttt aaa gca       344
Leu Asp Ile Ser His Ile Glu Gly Asp Ile Phe Ser Ser Phe Lys Ala
        85                  90                  95

ggt tat cca ttc tgg gga gcc ata ttt ttt tct att tct gga atg ttg       392
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Gly Tyr Pro Phe Trp Gly Ala Ile Phe Phe Ser Ile Ser Gly Met Leu
    100   105   110

tca att ata tct gaa agg aga aat gca aca tat ctg gtg aga gga agc 440
Ser Ile Ile Ser Glu Arg Arg Asn Ala Thr Tyr Leu Val Arg Gly Ser
115   120   125   130

ctg gga gca aac act gcc agc agc ata gct ggg gga acg gga att acc 488
Leu Gly Ala Asn Thr Ala Ser Ser Ile Ala Gly Gly Thr Gly Ile Thr
                135   140   145

atc ctg atc atc aac ctg aag aag agc ttg gcc tat atc cac atc cac 536
Ile Leu Ile Ile Asn Leu Lys Lys Ser Leu Ala Tyr Ile His Ile His
            150   155   160

agt tgc cag aaa ttt ttt gag acc aag tgc ttt atg gct tcc ttt tcc 584
Ser Cys Gln Lys Phe Phe Glu Thr Lys Cys Phe Met Ala Ser Phe Ser
        165   170   175

act gaa att gta gtg atg atg ctg ttt ctc acc att ctg gga ctt ggt 632
Thr Glu Ile Val Val Met Met Leu Phe Leu Thr Ile Leu Gly Leu Gly
    180   185   190

agt gct gtg tca ctc aca atc tgt gga gct ggg gaa gaa ctc aaa gga 680
Ser Ala Val Ser Leu Thr Ile Cys Gly Ala Gly Glu Glu Leu Lys Gly
195   200   205   210

aac aag gtt cca gag gat cgt gtt tat gaa gaa tta aac ata tat tca 728
Asn Lys Val Pro Glu Asp Arg Val Tyr Glu Glu Leu Asn Ile Tyr Ser
                215   220   225

gct act tac agt gag ttg gaa gac cca ggg gaa atg tct cct ccc att 776
Ala Thr Tyr Ser Glu Leu Glu Asp Pro Gly Glu Met Ser Pro Pro Ile
            230   235   240

gat tta taa gaatcacgtg tccagaacac tctgattcac agccaaggat 825
Asp Leu

ccagaaggcc aaggtcttgt taaggggcta ctggaaaaat ttctattctc tccacagcct 885

gctggtttt 894

<210> 42

<211> 244

<212> PRT

<213> Homo sapiens

<400> . 42

Met Asp Thr Glu Ser Asn Arg Arg Ala Asn Leu Ala Leu Pro Gln Glu
1   5   10   15

Pro Ser Ser Val Pro Ala Phe Glu Val Leu Glu Ile Ser Pro Gln Glu

20 25 30

Val Ser Ser Gly Arg Leu Leu Lys Ser Ala Ser Ser Pro Pro Leu His
35 40 45

Thr Trp Leu Thr Val Leu Lys Lys Glu Gln Glu Phe Leu Gly Val Thr
50 55 60

Gln Ile Leu Thr Ala Met Ile Cys Leu Cys Phe Gly Thr Val Val Cys
65 70 75 80

Ser Val Leu Asp Ile Ser His Ile Glu Gly Asp Ile Phe Ser Ser Phe
85 90 95

Lys Ala Gly Tyr Pro Phe Trp Gly Ala Ile Phe Phe Ser Ile Ser Gly
100 105 110

Met Leu Ser Ile Ile Ser Glu Arg Arg Asn Ala Thr Tyr Leu Val Arg
115 120 125

Gly Ser Leu Gly Ala Asn Thr Ala Ser Ser Ile Ala Gly Gly Thr Gly
130 135 140

Ile Thr Ile Leu Ile Ile Asn Leu Lys Lys Ser Leu Ala Tyr Ile His
145 150 155 160

Ile His Ser Cys Gln Lys Phe Phe Glu Thr Lys Cys Phe Met Ala Ser
165 170 175

Phe Ser Thr Glu Ile Val Val Met Met Leu Phe Leu Thr Ile Leu Gly
180 185 190

Leu Gly Ser Ala Val Ser Leu Thr Ile Cys Gly Ala Gly Glu Glu Leu
195 200 205

Lys Gly Asn Lys Val Pro Glu Asp Arg Val Tyr Glu Glu Leu Asn Ile
210 215 220

Tyr Ser Ala Thr Tyr Ser Glu Leu Glu Asp Pro Gly Glu Met Ser Pro
225 230 235 240

Pro Ile Asp Leu

<210> 43

<211> 1646

<212> DNA

<213> homo sapiens

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<300>

<301> Adra, CN, Lelias, JM, Kobayashi, H, Kaghad, M, Morrison, P, Rowley, JD, Lim, B

<302> Cloning of the cDNA for a hematopoietic cell-specific protein related to CD20 and the beta subunit of the high-affinity IgE receptor: evidence for a family of proteins with four membrane-spanning regions

<303> Proc Natl Acad Sci USA

<304> 91

<305> 21

<306> 10178-10182

<307> 1994-__-__

<309> _____-__-__

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tgccaaagcc tgaagcctcc aagccataaa caacccca atg gcc tcc cac gaa gtt     116
                                          Met Ala Ser His Glu Val
                                          1               5

gat aat gca gag ctg ggg tca gcc tct gcc cat ggt acc cca ggc agt       164
Asp Asn Ala Glu Leu Gly Ser Ala Ser Ala His Gly Thr Pro Gly Ser
            10                  15                  20

gag acg gga cca gaa gag ctg aat act tct gtc tac cac ccc ata aat       212
Glu Thr Gly Pro Glu Glu Leu Asn Thr Ser Val Tyr His Pro Ile Asn
        25                  30                  35
```

```
gga tca cca gat tat cag aaa gca aaa tta caa gtt ctt ggg gcc atc        260
Gly Ser Pro Asp Tyr Gln Lys Ala Lys Leu Gln Val Leu Gly Ala Ile
    40                  45                  50

cag atc ctg aat gca gca atg att ctg gct ttg ggt gtc ttt ctg ggt        308
Gln Ile Leu Asn Ala Ala Met Ile Leu Ala Leu Gly Val Phe Leu Gly
55                  60                  65                  70

tcc ttg caa tac cca tac cac ttc caa aag cac ttc ttt ttc ttc acc        356
Ser Leu Gln Tyr Pro Tyr His Phe Gln Lys His Phe Phe Phe Phe Thr
                75                  80                  85

ttc tac aca ggc tac ccg att tgg ggt gct gtg ttt ttc tgt agt tca        404
Phe Tyr Thr Gly Tyr Pro Ile Trp Gly Ala Val Phe Phe Cys Ser Ser
            90                  95                  100

gga acc ttg tct gtt gta gca ggg ata aaa ccc aca aga aca tgg ata        452
Gly Thr Leu Ser Val Val Ala Gly Ile Lys Pro Thr Arg Thr Trp Ile
        105                 110                 115

cag aac agt ttt gga atg aac att gcc agt gct aca att gca cta gtg        500
Gln Asn Ser Phe Gly Met Asn Ile Ala Ser Ala Thr Ile Ala Leu Val
    120                 125                 130

ggg act gct ttt ctc tca cta aat ata gca gtt aat atc cag tca tta        548
Gly Thr Ala Phe Leu Ser Leu Asn Ile Ala Val Asn Ile Gln Ser Leu
135                 140                 145                 150

agg agt tgt cac tct tca tca gag tca ccg gac cta tgc aat tac atg        596
Arg Ser Cys His Ser Ser Ser Glu Ser Pro Asp Leu Cys Asn Tyr Met
                155                 160                 165

ggc tcc ata tca aat ggc atg gtg tct cta ctg ctg att ctc acc ttg        644
Gly Ser Ile Ser Asn Gly Met Val Ser Leu Leu Leu Ile Leu Thr Leu
            170                 175                 180

ctg gaa tta tgc gta act atc tct acc ata gcc atg tgg tgc aat gca        692
Leu Glu Leu Cys Val Thr Ile Ser Thr Ile Ala Met Trp Cys Asn Ala
        185                 190                 195

aac tgc tgt aat tca aga gag gaa att tcc tca cct ccc aat tct gtg        740
Asn Cys Cys Asn Ser Arg Glu Glu Ile Ser Ser Pro Pro Asn Ser Val
    200                 205                 210

taa tcaagaatac ctccttatga aaataattct gagagcatga atatttgacc             793

ttaaatctcc agtgactcag agcttcaccc acaaactcag gagaacataa gcctgctcgt      853

aaagctcaat ccttctatca tggcaccaat cacaagaacc ttggacgttt gactgactct      913

atcctttctc tcctaactat aaatcctatt tgtgtgtcgt gggtatggaa ggacagatat      973

atttctttag gcattcttgg atatctgtaa cttctatgat cattactcca aagttgtttc     1033

cagaaattgg ttctatttct tcttatccac ctactccatt gctttatgag gtttaaggaa     1093

ggaaggcggt ataatcccta ttcaatatat tttttctaaa atccaacttc tgaccgccca     1153

gtaggaagaa aaatgagaca ttttttccat tacagagaaa tgcttcttga ctttaacatc     1213
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agcattataa aaagtgtcaa ataaaaaatt accatcatta tcattaaaat aaattttcac 1273

tgtatttgag atgggagggt taaggctcag ggattttatt tcagtgaact gctggaactc 1333

acacatgccc tgatatgtaa atgatgattt atgttggcga gtctgagagc aagcccaaat 1393

gtgttcttca aaggacaatg ggaaactgta aagtagagaa ctaaagaata aggcctttag 1453

aatctgacac atctgggttc aaattctgaa actgtcactt attacctgta tgaacatggg 1513

caaattatct aatctctctg atctattttt cctcatctgt aaaataggtg taataataac 1573

aactactttg tcggttgctc tgagggttaa atgaaaataa aaagaaaatg tgaaacagca 1633

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<211> 214

<212> PRT

<213> homo sapiens

<400> 44

Met Ala Ser His Glu Val Asp Asn Ala Glu Leu Gly Ser Ala Ser Ala
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His Gly Thr Pro Gly Ser Glu Thr Gly Pro Glu Glu Leu Asn Thr Ser
            20   25   30

Val Tyr His Pro Ile Asn Gly Ser Pro Asp Tyr Gln Lys Ala Lys Leu
        35   40   45

Gln Val Leu Gly Ala Ile Gln Ile Leu Asn Ala Ala Met Ile Leu Ala
    50   55   60

Leu Gly Val Phe Leu Gly Ser Leu Gln Tyr Pro Tyr His Phe Gln Lys
65   70   75   80

His Phe Phe Phe Phe Thr Phe Tyr Thr Gly Tyr Pro Ile Trp Gly Ala
                85   90   95

Val Phe Phe Cys Ser Ser Gly Thr Leu Ser Val Val Ala Gly Ile Lys
            100   105   110

Pro Thr Arg Thr Trp Ile Gln Asn Ser Phe Gly Met Asn Ile Ala Ser
        115   120   125

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Ala Thr Ile Ala Leu Val Gly Thr Ala Phe Leu Ser Leu Asn Ile Ala
    130                 135                 140

Val Asn Ile Gln Ser Leu Arg Ser Cys His Ser Ser Ser Glu Ser Pro
145                 150                 155                 160

Asp Leu Cys Asn Tyr Met Gly Ser Ile Ser Asn Gly Met Val Ser Leu
                165                 170                 175

Leu Leu Ile Leu Thr Leu Leu Glu Leu Cys Val Thr Ile Ser Thr Ile
            180                 185                 190

Ala Met Trp Cys Asn Ala Asn Cys Cys Asn Ser Arg Glu Glu Ile Ser
        195                 200                 205

Ser Pro Pro Asn Ser Val
    210

<210>  45

<211>  1182

<212>  DNA

<213>  Homo sapiens

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<221>  cDNA

<222>  (1)..(1182)

<220>

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<222>  (58)..(861)

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atg atg tca tcc aag cca aca agc cat gct gaa gta aat gaa acc ata     105
Met Met Ser Ser Lys Pro Thr Ser His Ala Glu Val Asn Glu Thr Ile
1               5                   10                  15

ccc aac cct tac cca cca ggc agc ttt atg gct cct gga ttt caa cag     153
Pro Asn Pro Tyr Pro Pro Gly Ser Phe Met Ala Pro Gly Phe Gln Gln
            20                  25                  30
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```
cct ctg ggt tca atc aac tta gaa aac caa gct cag ggt gct cag cgt      201
Pro Leu Gly Ser Ile Asn Leu Glu Asn Gln Ala Gln Gly Ala Gln Arg
        35                  40                  45

gct cag ccc tac ggc atc aca tct ccg gga atc ttt gct agc agt caa      249
Ala Gln Pro Tyr Gly Ile Thr Ser Pro Gly Ile Phe Ala Ser Ser Gln
    50                  55                  60

ccg ggt caa gga aat ata caa atg ata aat cca agt gtg gga aca gca      297
Pro Gly Gln Gly Asn Ile Gln Met Ile Asn Pro Ser Val Gly Thr Ala
65                  70                  75                  80

gta atg aac ttt aaa gaa gaa gca aag gca cta ggg gtg atc cag atc      345
Val Met Asn Phe Lys Glu Glu Ala Lys Ala Leu Gly Val Ile Gln Ile
                85                  90                  95

atg gtt gga ttg atg cac att ggt ttt gga att gtt ttg tgt tta ata      393
Met Val Gly Leu Met His Ile Gly Phe Gly Ile Val Leu Cys Leu Ile
            100                 105                 110

tcc ttc tct ttt aga gaa gta tta ggt ttt gcc tct act gct gtt att      441
Ser Phe Ser Phe Arg Glu Val Leu Gly Phe Ala Ser Thr Ala Val Ile
        115                 120                 125

ggt gga tac cca ttc tgg ggt ggc ctt tct ttt att atc tct ggc tct      489
Gly Gly Tyr Pro Phe Trp Gly Gly Leu Ser Phe Ile Ile Ser Gly Ser
    130                 135                 140

ctc tct gtg tca gca tcc aag gag ctt tcc cgt tgt ctg gtg aaa ggc      537
Leu Ser Val Ser Ala Ser Lys Glu Leu Ser Arg Cys Leu Val Lys Gly
145                 150                 155                 160

agc ctg gga atg aac att gtt agt tct atc ttg gcc ttc att gga gtg      585
Ser Leu Gly Met Asn Ile Val Ser Ser Ile Leu Ala Phe Ile Gly Val
                165                 170                 175

att ctg ctg ctg gtg gat atg tgc atc aat ggg gta gct ggc caa gac      633
Ile Leu Leu Leu Val Asp Met Cys Ile Asn Gly Val Ala Gly Gln Asp
            180                 185                 190

tac tgg gcc gtg ctt tct gga aaa ggc att tca gcc acg ctg atg atc      681
Tyr Trp Ala Val Leu Ser Gly Lys Gly Ile Ser Ala Thr Leu Met Ile
        195                 200                 205

ttc tcc ctc ttg gag ttc ttc gta gct tgt gcc aca gcc cat ttt gcc      729
Phe Ser Leu Leu Glu Phe Phe Val Ala Cys Ala Thr Ala His Phe Ala
    210                 215                 220

aac caa gca aac acc aca acc aat atg tct gtc ctg gtt att cca aat      777
Asn Gln Ala Asn Thr Thr Thr Asn Met Ser Val Leu Val Ile Pro Asn
225                 230                 235                 240

atg tat gaa agc aac cct gtg aca cca gcg tct tct tca gct cct ccc      825
Met Tyr Glu Ser Asn Pro Val Thr Pro Ala Ser Ser Ser Ala Pro Pro
                245                 250                 255

aga tgc aac aac tac tca gct aat gcc cct aaa tag taaaagaaaa           871
Arg Cys Asn Asn Tyr Ser Ala Asn Ala Pro Lys
            260                 265
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aggggtatca gtctaatctc atggagaaaa actacttgca aaaacttctt aagaagatgt 931

cttttattgt ctacaatgat ttctagtctt taaaaactgt gtttgagatt tgtttttagg 991

ttggtcgcta atgatggctg tatctccctt cactgtctct tcctacatta ccactactac 1051

atgctggcaa aggtgaagga tcagaggact gaaaaatgat tctgcaactc tcttaaagtt 1111

agaaatgttt ctgttcatat tactttttcc ttaataaaat gtcattagaa acaaaaaaaa 1171

aaaaaaaaaa a 1182

<210> 46

<211> 267

<212> PRT

<213> Homo sapiens

<400> 46

Met Met Ser Ser Lys Pro Thr Ser His Ala Glu Val Asn Glu Thr Ile
1 5 10 15

Pro Asn Pro Tyr Pro Pro Gly Ser Phe Met Ala Pro Gly Phe Gln Gln
20 25 30

Pro Leu Gly Ser Ile Asn Leu Glu Asn Gln Ala Gln Gly Ala Gln Arg
35 40 45

Ala Gln Pro Tyr Gly Ile Thr Ser Pro Gly Ile Phe Ala Ser Ser Gln
50 55 60

Pro Gly Gln Gly Asn Ile Gln Met Ile Asn Pro Ser Val Gly Thr Ala
65 70 75 80

Val Met Asn Phe Lys Glu Glu Ala Lys Ala Leu Gly Val Ile Gln Ile
85 90 95

Met Val Gly Leu Met His Ile Gly Phe Gly Ile Val Leu Cys Leu Ile
100 105 110

Ser Phe Ser Phe Arg Glu Val Leu Gly Phe Ala Ser Thr Ala Val Ile
115 120 125

Gly Gly Tyr Pro Phe Trp Gly Gly Leu Ser Phe Ile Ile Ser Gly Ser
130 135 140

```
Leu Ser Val Ser Ala Ser Lys Glu Leu Ser Arg Cys Leu Val Lys Gly
145                 150                 155                 160

Ser Leu Gly Met Asn Ile Val Ser Ser Ile Leu Ala Phe Ile Gly Val
                165                 170                 175

Ile Leu Leu Leu Val Asp Met Cys Ile Asn Gly Val Ala Gly Gln Asp
            180                 185                 190

Tyr Trp Ala Val Leu Ser Gly Lys Gly Ile Ser Ala Thr Leu Met Ile
        195                 200                 205

Phe Ser Leu Leu Glu Phe Phe Val Ala Cys Ala Thr Ala His Phe Ala
    210                 215                 220

Asn Gln Ala Asn Thr Thr Thr Asn Met Ser Val Leu Val Ile Pro Asn
225                 230                 235                 240

Met Tyr Glu Ser Asn Pro Val Thr Pro Ala Ser Ser Ser Ala Pro Pro
                245                 250                 255

Arg Cys Asn Asn Tyr Ser Ala Asn Ala Pro Lys
            260                 265
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<210> 47

<211> 1388

<212> DNA

<213> Mus musculus

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<221> cDNA

<222> (1)..(1388)

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<221> CDS

<222> (178)..(1053)

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<301> Tedder, TF, Klejman, G, Disteche, CM, Adler, DA, Schlossman, SF, Saito, H

<302> Cloning of complementary DNA encoding a new mouse B lymphocyte differentiation antigen, homologous to the human B1 (CD20) antigen and localization of the gen to chromosome 19

<303> J Immunol

<304> 141

<305> 12

<306> 4388-4394

<307> 1988-__-__

<309> ____-__-__

<400> 47

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gaattccttt tttttttttt tttttaaaga tttatttatt attatatgta agtacactgt      60

agctatcttc aagtacttga gatagaagag gccaactgat ctcagctgtg agtggctaat     120

ttggccctta agccttggag ccttggagcc ttggagaccc aggcgtttga aaactca        177

atg agt gga cct ttc cca gca gag cct aca aaa ggt ccc ctc gcc atg       225
Met Ser Gly Pro Phe Pro Ala Glu Pro Thr Lys Gly Pro Leu Ala Met
1               5                   10                  15

caa cct gct cca aaa gtg aac ctc aaa agg aca tct tca ctg gtg ggc       273
Gln Pro Ala Pro Lys Val Asn Leu Lys Arg Thr Ser Ser Leu Val Gly
            20                  25                  30

ccc aca caa agc ttc ttc atg agg gaa tca aag gct ttg ggg gct gtc       321
Pro Thr Gln Ser Phe Phe Met Arg Glu Ser Lys Ala Leu Gly Ala Val
        35                  40                  45

caa atc atg aat ggc ctc ttc cat att acc ctg ggg gga ctg ctg atg       369
Gln Ile Met Asn Gly Leu Phe His Ile Thr Leu Gly Gly Leu Leu Met
    50                  55                  60

atc ccc aca ggg gtc ttc gca ccc atc tgt ttg agt gta tgg tac cct       417
Ile Pro Thr Gly Val Phe Ala Pro Ile Cys Leu Ser Val Trp Tyr Pro
65                  70                  75                  80

ctc tgg gga ggc att atg tac att att tca gga tca ctc ctg gca gct       465
Leu Trp Gly Gly Ile Met Tyr Ile Ile Ser Gly Ser Leu Leu Ala Ala
                85                  90                  95

gca gca gaa aaa acc tcc agg aag agt ttg gtc aaa gca aaa gtg ata       513
Ala Ala Glu Lys Thr Ser Arg Lys Ser Leu Val Lys Ala Lys Val Ile
            100                 105                 110

atg agc tct cta agc ctc ttt gct gcc att tct gga ata att ctt tca       561
Met Ser Ser Leu Ser Leu Phe Ala Ala Ile Ser Gly Ile Ile Leu Ser
        115                 120                 125
```

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atc atg gac ata ctt aac atg aca ctt tct cat ttt tta aaa atg aga      609
Ile Met Asp Ile Leu Asn Met Thr Leu Ser His Phe Leu Lys Met Arg
    130                 135                 140

aga ctg gag ctt att caa act tcc aag ccg tat gtt gat atc tac gac      657
Arg Leu Glu Leu Ile Gln Thr Ser Lys Pro Tyr Val Asp Ile Tyr Asp
145                 150                 155                 160

tgt gaa cca tct aat tcc tca gag aaa aac tcc cca tct aca cag tac      705
Cys Glu Pro Ser Asn Ser Ser Glu Lys Asn Ser Pro Ser Thr Gln Tyr
                165                 170                 175

tgt aac agc att cag tct gtg ttc ttg ggc att ctg tcg gcg atg ctg      753
Cys Asn Ser Ile Gln Ser Val Phe Leu Gly Ile Leu Ser Ala Met Leu
            180                 185                 190

atc tct gcc ttc ttc cag aaa ctt gtg aca gct ggt att gtg gag aat      801
Ile Ser Ala Phe Phe Gln Lys Leu Val Thr Ala Gly Ile Val Glu Asn
        195                 200                 205

gag tgg aaa aga atg tgt acc aga tcc aaa tct aat gtg gtt ctg ctg      849
Glu Trp Lys Arg Met Cys Thr Arg Ser Lys Ser Asn Val Val Leu Leu
    210                 215                 220

tca gct gga gaa aaa aat gag cag acg att aaa atg aaa gaa gaa atc      897
Ser Ala Gly Glu Lys Asn Glu Gln Thr Ile Lys Met Lys Glu Glu Ile
225                 230                 235                 240

att gag cta agt gga gta tct tcc caa cca aag aat gaa gag gaa att      945
Ile Glu Leu Ser Gly Val Ser Ser Gln Pro Lys Asn Glu Glu Glu Ile
                245                 250                 255

gaa att att cca gtg cag gag gaa gaa gaa gaa gaa gca gaa ata aat      993
Glu Ile Ile Pro Val Gln Glu Glu Glu Glu Glu Glu Ala Glu Ile Asn
            260                 265                 270

ttt cca gca cct ccc caa gag cag gaa tcc ttg cca gtg gaa aat gag     1041
Phe Pro Ala Pro Pro Gln Glu Gln Glu Ser Leu Pro Val Glu Asn Glu
        275                 280                 285

atc gct cct taa actcttttct tttctaagca ttattgttta gagagcttcc         1093
Ile Ala Pro
    290

aagacacata gttaccctca tctcttgtgg ccttccacaa tctattctcc atattttcac   1153

agcttaactt tgcatagaga agccacatct agctctcctt cacatttgaa gaatgcagtg   1213

attataaaag attgtctttt gccttgctta gggagtctta cactggcaga aacgctgaag   1273

aatccaattc tcattcacct tttccttgga tgtgtgtctc agtagtggta atggtttttc   1333

cgcatttcct ccatcagcag ttacagcaga aggagtcaga gagttcaggg aattc        1388
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<210> 48

<211> 291

<212> PRT

<213> Mus musculus

<400> 48

Met Ser Gly Pro Phe Pro Ala Glu Pro Thr Lys Gly Pro Leu Ala Met
1 5 10 15

Gln Pro Ala Pro Lys Val Asn Leu Lys Arg Thr Ser Ser Leu Val Gly
20 25 30

Pro Thr Gln Ser Phe Phe Met Arg Glu Ser Lys Ala Leu Gly Ala Val
35 40 45

Gln Ile Met Asn Gly Leu Phe His Ile Thr Leu Gly Gly Leu Leu Met
50 55 60

Ile Pro Thr Gly Val Phe Ala Pro Ile Cys Leu Ser Val Trp Tyr Pro
65 70 75 80

Leu Trp Gly Gly Ile Met Tyr Ile Ile Ser Gly Ser Leu Leu Ala Ala
85 90 95

Ala Ala Glu Lys Thr Ser Arg Lys Ser Leu Val Lys Ala Lys Val Ile
100 105 110

Met Ser Ser Leu Ser Leu Phe Ala Ala Ile Ser Gly Ile Ile Leu Ser
115 120 125

Ile Met Asp Ile Leu Asn Met Thr Leu Ser His Phe Leu Lys Met Arg
130 135 140

Arg Leu Glu Leu Ile Gln Thr Ser Lys Pro Tyr Val Asp Ile Tyr Asp
145 150 155 160

Cys Glu Pro Ser Asn Ser Ser Glu Lys Asn Ser Pro Ser Thr Gln Tyr
165 170 175

Cys Asn Ser Ile Gln Ser Val Phe Leu Gly Ile Leu Ser Ala Met Leu
180 185 190

Ile Ser Ala Phe Phe Gln Lys Leu Val Thr Ala Gly Ile Val Glu Asn
195 200 205

Glu Trp Lys Arg Met Cys Thr Arg Ser Lys Ser Asn Val Val Leu Leu

210 215 220

Ser Ala Gly Glu Lys Asn Glu Gln Thr Ile Lys Met Lys Glu Glu Ile
225 230 235 240

Ile Glu Leu Ser Gly Val Ser Ser Gln Pro Lys Asn Glu Glu Glu Ile
245 250 255

Glu Ile Ile Pro Val Gln Glu Glu Glu Glu Glu Glu Ala Glu Ile Asn
260 265 270

Phe Pro Ala Pro Pro Gln Glu Gln Glu Ser Leu Pro Val Glu Asn Glu
275 280 285

Ile Ala Pro
290

<210> 49

<211> 1010

<212> DNA

<213> Mus musculus

<220>

<221> cDNA

<222> (1)..(1010)

<220>

<221> CDS

<222> (23)..(730)

<300>

<301> Ra, C, Jouvin, M-He, Kinet, J-P

<302> Complete structure of the mouse mast cell receptor for IgE (FceRI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells

<303> J Biol Chem

<304> 264

<305> 26

<306> 15323-15327

<307> 1989-__-__

<309> ____-__-__

<400> 49

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                         Met Asp Thr Glu Asn Arg Ser Arg Ala Asp
                         1               5                   10

ctt gct ctc cca aat cca caa gaa tcc tcc agt gca cct gac att gaa       100
Leu Ala Leu Pro Asn Pro Gln Glu Ser Ser Ser Ala Pro Asp Ile Glu
                15                  20                  25

ctc ttg gaa gca tct cct gcc aaa gca gcc cca cca aag cag aca tgg       148
Leu Leu Glu Ala Ser Pro Ala Lys Ala Ala Pro Pro Lys Gln Thr Trp
            30                  35                  40

cgg aca ttt ttg aag aaa gag ttg gag ttc ctg gga gca aca caa att       196
Arg Thr Phe Leu Lys Lys Glu Leu Glu Phe Leu Gly Ala Thr Gln Ile
        45                  50                  55

ctg gtt ggt ttg ata tgc ctt tgt ttt gga aca att gtc tgc tcc gta       244
Leu Val Gly Leu Ile Cys Leu Cys Phe Gly Thr Ile Val Cys Ser Val
    60                  65                  70

ctc tat gtt tca gac ttt gat gaa gaa gtg ctt tta ctt tat aaa cta       292
Leu Tyr Val Ser Asp Phe Asp Glu Glu Val Leu Leu Leu Tyr Lys Leu
75                  80                  85                  90

ggc tat cca ttc tgg ggt gca gtg ctg ttt gtt ttg tct gga ttt ttg       340
Gly Tyr Pro Phe Trp Gly Ala Val Leu Phe Val Leu Ser Gly Phe Leu
                95                  100                 105

tca att atc tcc gaa aga aaa aac aca ttg tat ctg gtg aga ggc agc       388
Ser Ile Ile Ser Glu Arg Lys Asn Thr Leu Tyr Leu Val Arg Gly Ser
            110                 115                 120

ctg gga gca aac att gtc agt agc atc gct gca ggg acg ggg atc gcc       436
Leu Gly Ala Asn Ile Val Ser Ser Ile Ala Ala Gly Thr Gly Ile Ala
        125                 130                 135

atg ctg atc ctc aat ctg acc aat aac ttc gct tat atg aac aac tgc       484
Met Leu Ile Leu Asn Leu Thr Asn Asn Phe Ala Tyr Met Asn Asn Cys
    140                 145                 150

aag aat gta acc gaa gac gac ggc tgc ttt gtg gct tct ttc acc aca       532
Lys Asn Val Thr Glu Asp Asp Gly Cys Phe Val Ala Ser Phe Thr Thr
155                 160                 165                 170

gaa ctg gtg ttg atg atg ctg ttt ctc acc atc ctg gcc ttt tgc agt       580
Glu Leu Val Leu Met Met Leu Phe Leu Thr Ile Leu Ala Phe Cys Ser
                175                 180                 185

gct gtg ttg ttc act atc tat agg att gga caa gag tta gaa agt aaa       628
Ala Val Leu Phe Thr Ile Tyr Arg Ile Gly Gln Glu Leu Glu Ser Lys
```

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            190                 195                 200

aag gtc cca gat gat cgt ctt tat gaa gaa tta aat gtg tat tca cca       676
Lys Val Pro Asp Asp Arg Leu Tyr Glu Glu Leu Asn Val Tyr Ser Pro
        205                 210                 215

att tac agt gag ttg gaa gac aaa ggg gaa aca tct tct cca gtt gat       724
Ile Tyr Ser Glu Leu Glu Asp Lys Gly Glu Thr Ser Ser Pro Val Asp
    220                 225                 230

tca taa gaatcagggg accaggacaa tctgattcaa gtataatctt gaaagttgat       780
Ser
235

ctttttacaa aattctcgca aaatttctgt ttgttccaca ttctgtcagt ttttcaattg    840

gattgttctg cagatgccac tcttttagtt atgctgtatc tgatcttcta aatatctccc    900

tttttgcgga tatcattcac tccaattttc ttgttttgtg tcacaatttc acatacatct    960

tttctggaaa gtcatcaagg aataagttgg ctttattgta tgtctacttt              1010


<210>  50

<211>  235

<212>  PRT

<213>  Mus musculus


<400>  50

Met Asp Thr Glu Asn Arg Ser Arg Ala Asp Leu Ala Leu Pro Asn Pro
1               5                   10                  15


Gln Glu Ser Ser Ser Ala Pro Asp Ile Glu Leu Leu Glu Ala Ser Pro
            20                  25                  30


Ala Lys Ala Ala Pro Pro Lys Gln Thr Trp Arg Thr Phe Leu Lys Lys
        35                  40                  45


Glu Leu Glu Phe Leu Gly Ala Thr Gln Ile Leu Val Gly Leu Ile Cys
    50                  55                  60


Leu Cys Phe Gly Thr Ile Val Cys Ser Val Leu Tyr Val Ser Asp Phe
65                  70                  75                  80


Asp Glu Glu Val Leu Leu Leu Tyr Lys Leu Gly Tyr Pro Phe Trp Gly
                85                  90                  95


Ala Val Leu Phe Val Leu Ser Gly Phe Leu Ser Ile Ile Ser Glu Arg
            100                 105                 110
```

```
Lys Asn Thr Leu Tyr Leu Val Arg Gly Ser Leu Gly Ala Asn Ile Val
        115                 120                 125

Ser Ser Ile Ala Ala Gly Thr Gly Ile Ala Met Leu Ile Leu Asn Leu
    130                 135                 140

Thr Asn Asn Phe Ala Tyr Met Asn Asn Cys Lys Asn Val Thr Glu Asp
145                 150                 155                 160

Asp Gly Cys Phe Val Ala Ser Phe Thr Thr Glu Leu Val Leu Met Met
                165                 170                 175

Leu Phe Leu Thr Ile Leu Ala Phe Cys Ser Ala Val Leu Phe Thr Ile
            180                 185                 190

Tyr Arg Ile Gly Gln Glu Leu Glu Ser Lys Lys Val Pro Asp Asp Arg
        195                 200                 205

Leu Tyr Glu Glu Leu Asn Val Tyr Ser Pro Ile Tyr Ser Glu Leu Glu
    210                 215                 220

Asp Lys Gly Glu Thr Ser Ser Pro Val Asp Ser
225                 230                 235
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<210> 51

<211> 2542

<212> DNA

<213> rat

<220>

<221> cDNA

<222> (1)..(2542)

<400> 51

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cctgacattg aactcttgga agcgtcccct cctgcaaaag ctctaccaga gaagccagcc     180

tcacccccac cacagcagac atggcagtca tttttgaaga aagagttgga gttcctgggc     240
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gtaacccaag ttctggttgg tttgatatgc ctttgttttg gaacagttgt ctgctccaca 300
ctccagactt cagactttga cgacgaagtg cttttattat atagagcagg ctacccattc 360
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tcataagaat caaggggcca ggacaatctg attccagtat agtcttgaga gtcgatcttt 840
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taactaccaa caattggtag agattgccat gtatgcctaa aatctcccag cccaaggcca 1620
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caaatgtacc tagagagcaa acctgaatag gaggtaaaat gggggaaaag cagctaagaa 2280

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atcttcctgt aaggctcacc tcatatgtct cttcacatag ccacaccctt gattcacagt 2460

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ataaaataaa tcagaattat tt 2542

<210> 52

<211> 243

<212> PRT

<213> rat

<400> 52

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Gln Glu Ser Pro Ser Ala Pro Asp Ile Glu Leu Leu Glu Ala Ser Pro
20 25 30

Pro Ala Lys Ala Leu Pro Glu Lys Pro Ala Ser Pro Pro Pro Gln Gln
35 40 45

Thr Trp Gln Ser Phe Leu Lys Lys Glu Leu Glu Phe Leu Gly Val Thr
50 55 60

Gln Val Leu Val Gly Leu Ile Cys Leu Cys Phe Gly Thr Val Val Cys
65 70 75 80

Ser Thr Leu Gln Thr Ser Asp Phe Asp Asp Glu Val Leu Leu Leu Tyr
85 90 95

Arg Ala Gly Tyr Pro Phe Trp Gly Ala Val Leu Phe Val Leu Ser Gly
100 105 110

Phe Leu Ser Ile Met Ser Glu Arg Lys Asn Thr Leu Tyr Leu Val Arg
115 120 125

Gly Ser Leu Gly Ala Asn Ile Val Ser Ser Ile Ala Ala Gly Leu Gly
130 135 140

Ile Ala Ile Leu Ile Leu Asn Leu Ser Asn Asn Ser Ala Tyr Met Asn
145 150 155 160

Tyr Cys Lys Asp Ile Thr Glu Asp Asp Gly Cys Phe Val Thr Ser Phe
165 170 175

Ile Thr Glu Leu Val Leu Met Leu Leu Phe Leu Thr Ile Leu Ala Phe
180 185 190

Cys Ser Ala Val Leu Leu Ile Ile Tyr Arg Ile Gly Gln Glu Phe Glu
195 200 205

Arg Ser Lys Val Pro Asp Asp Arg Leu Tyr Glu Glu Leu His Val Tyr
210 215 220

Ser Pro Ile Tyr Ser Ala Leu Glu Asp Thr Arg Glu Ala Ser Ala Pro
225 230 235 240

Val Val Ser

<210> 53

<211> 1323

<212> DNA

<213> dog

<220>

<221> cDNA

<222> (1)..(1323)

<400> 53

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cctgaaattg aactttcaga agtacctctc cgtgaaaacc ccttactgga gaaggctggt 180

tcatccccac cgtgccatac gtggctgatg tttctgaaga gggagctgga atttctgggg 240

gtaacacaaa ttctgattgc cttggtatgt ctttgttttg gaataattgt ctgctccgtg 300

atcaatattt cagaatttaa ggaagacatt ttttcatcat ttaaagcagg ctacccattc 360

tggggagcag tgtttttgc tatttctgga tttttgccaa ttatgtctga aaagaaacat 420

gctacatatc tggcgtgggg cagcctggga gccaacactg tccagatagc tgcgggaata 480

ggaatcttta tctacggtca acctgcagac ctgcagagga gctcggctta catccacaac 540

tgccagcaag ctcccagaga cgacttctgc tttgtggctt gtttttccac agaaattgtg 600

gcaatgatcc tgtttctcac tattctgggg ttttgcagtg ctgtgtcact gacgatctat 660

ggagttggag aattagtcca agggaacaag attgtagaag atcgtcttta tgaagaatta 720

aacatatatt caccgattta cagtgagctg gaagacaaag cggaggaatc ttctcctgtt 780

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Ala Gly Tyr Trp Gly Ala Val Ala Ser Gly Met Ser Lys Lys His Ala
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Thr Tyr Ala Trp Gly Ser Gly Ala Asn Thr Val Ala Ala Gly Gly Tyr
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